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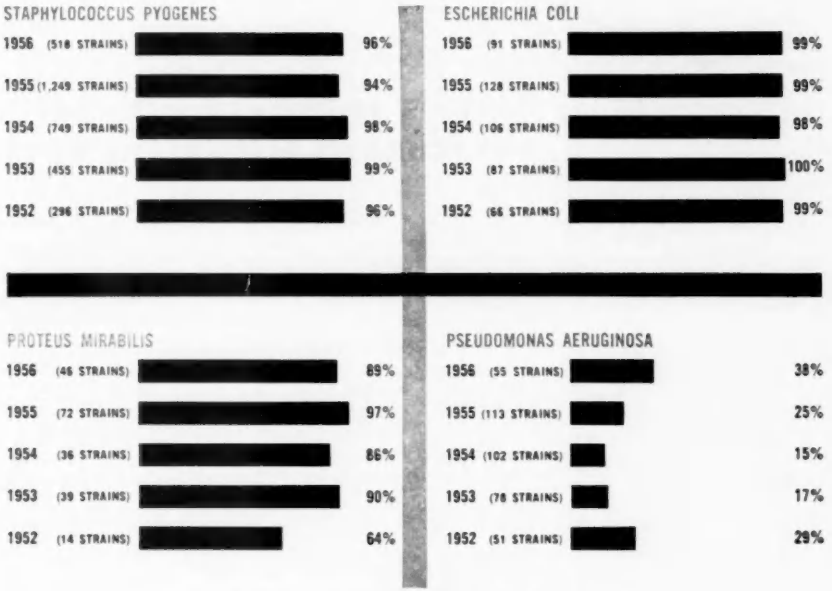

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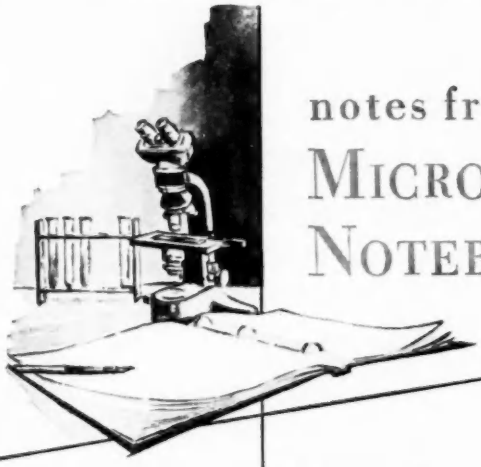

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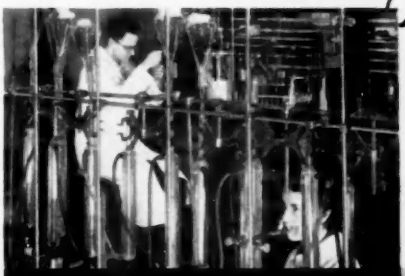

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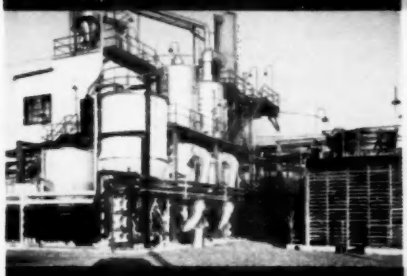

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INTRODUCTION TO THE 22ND CRAIG LECTURER, DR. L. L. WILLIAMS, JR.

JUSTIN M. ANDREWS

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda 14, Md.

For the benefit of the younger and recently acquired members of the Society, who may not be well acquainted with the life and achievements of the figure for whom this lecture series is named, I have the following information to offer.

Colonel Charles Franklin Craig, U. S. Army, was born in Danbury, Conn., on the fourth of July, 1872, the son of a country doctor. He obtained his medical degree at Yale in 1894, practiced medicine in his home town, and became a contract surgeon in the U. S. Army in 1898. Five years later he was commissioned as a First Lieutenant in what was then known as the Army Medical Corps, receiving his colonelcy in 1918. He served in Manila, Hawaii, Texas, Kansas, and the District of Columbia, where he had extended tours of duty at the Army Medical School. He made and reported numerous observations on tropical and related diseases in the field and had much to do with developing the laboratory technology of tropical medicine. He retired from the Army in 1931 and became Professor of Tropical Medicine and Head of that Department at Tulane Medical School until 1937, when he retired for the second time and went to live in San Antonio, Texas. He died in 1950.

Those of you desiring to refresh your memory further about his biography should consult the article entitled "Fifty Years of Work and Service in Tropical Medicine" by Dr. C. C. Bass at Tulane. This was presented by Dr. Bass as the 16th Craig Lecture at the Chicago meeting of the Society. It is the lead article in the first number of the American Journal of Tropical Medicine and Hygiene, which is the Commemorative Issue in Honor of Charles Franklin Craig. This was published in January 1952, following the amalgamation of the American Society of Tropical Medicine and the National Malaria Society.

Colonel Craig had a powerful impact on tropical medicine in this country. He was an inspiring teacher and a prodigious writer, authoring some 226 titles. He wrote seven textbooks and contributed sections to eleven others; the remainder of his publications are original articles. His early scientific interests were in the field of bacteriology, but he became successively concerned with the insect-transmitted diseases, especially malaria, and finally with amebiasis. He devised a well-known immunologic method of diagnosis for the latter. He was the Editor of the American Journal of Tropical Medicine from 1927 to 1946.

The Transactions of the American Society of Tropical Medicine of 1935 contain the cryptic authorization by the Council for the President to appoint a committee "to consider and report recommendations on the matter of a special, named lecture, with or without honorarium, to be given annually in the scientific program of the Society." President Vedder appointed Drs. Boyd, Clark, and Reed, and the following year the series of Charles Franklin Craig lectures was inaugurated by Dr. Ernest Muir, of London, England. According to the Transactions of the Society for that year, "Remarks" were made by Dr. Meleney to Dr. Muir and Colonel Craig on the inauguration of the lecture in honor of Colonel Craig. These were responded to by the latter but, unfortunately, none of this exchange was published. The names of the succeeding lecturers in the series constitute a veritable "Who's Who" of Tropical Medicine. They are listed below, together with the titles of the material presented.

First. Dr. Ernest Muir, London, England. The control of leprosy. *Am. J. Trop. Med.*, **17:** 51–58, 1937.

Second. Dr. George W. McCoy, Washington, D. C. History of leprosy in the United States. *Am. J. Trop Med.*, **18:** 19–35, 1938.

Third. Dr. Richard P. Strong, Boston, Mass. Progress in the study of infections due to Bartonella and Rickettsia. *Am. J. Trop. Med.*, **20:** 13–47, 1940.

Fourth. Dr. William H. Taliaferro, Chicago, Ill. The mechanism of acquired immunity to metazoal parasites. *Am. J. Trop. Med.*, **20:** 169–183, 1940.

Fifth. Dr. R. E. Dyer, Bethesda, Maryland. The control of typhus fever. *Am. J. Trop. Med.*, **21:** 163–185, 1941.

Sixth. Dr. K. F. Meyer, San Francisco, Cal. The known and unknown in plague. *Am. J. Trop. Med.*, **22:** 9–37, 1942.

Seventh. Rear Adm. Ross T. McIntire, M.C., U. S. Navy, Washington, D. C. The importance of tropical medicine in the armed forces. *Am. J. Trop. Med.*, **23:** 141–147, 1943.

Eighth. Col. George R. Callender, M.C., U. S. Army, Washington, D. C. Diarrheal diseases. *Am. J. Trop. Med.*, **24:** 7–17, 1944.

Ninth. Capt. L. T. Coggeshall, M.C., U. S. Navy Reserve, Klamath Falls, Oregon. Malaria and filariasis in the returning serviceman. *Am. J. Trop. Med.*, **25:** 177–185, 1945.

Tenth. Col. Paul F. Russell, M.C., A. U. S., Washington, D. C. Lessons in malariology from World War II. *Am. J. Trop. Med.*, **26:** 5–15, 1946.

Eleventh. Capt. James J. Sapero, M.C., U. S. Navy, Washington, D. C. New concepts in the treatment of relapsing malaria. *Am. J. Trop. Med.*, **27:** 271–285, 1947.

Twelfth. Dr. W. McD. Hammon, San Francisco, Cal. The arthropod-borne virus encephalitides. *Am. J. Trop. Med.*, **28:** 515–527, 1948.

Thirteenth. Dr. Herbert C. Clark, Panama, R. de P. The tropics and the white man. *Am. J. Trop. Med.*, **29:** 303–311, 1949.

Fourteenth. Dr. Joseph E. Smadel, Washington, D. C. Antibiotics in typhus and typhoid fevers. *Am. J. Trop. Med.*, **30:** 357–363, 1950.

Fifteenth. Dr. William J. Darby, Nashville, Tenn. Advances in the therapy of nutritional deficiencies. *Am. J. Trop. Med.*, **31:** 183–192, 1951.

Sixteenth. Dr. C. C. Bass, New Orleans, La. Charles Franklin Craig: Fifty years of work and service in tropical medicine. *Am. J. Trop. Med. & Hyg.*, **1:** 5–20, 1952.

Seventeenth. Dr. Abel Wolman, Baltimore, Md. Financing sanitary works in the tropics—A challenge. *Am. J. Trop. Med. & Hyg.*, **2:** 557–565, 1953.

Eighteenth. Dr. Henry E. Meleney, New Orleans, La. Problems in the control of schistosomiasis. *Am. J. Trop. Med. & Hyg.*, **3:** 209–219, 1954.

Nineteenth. Dr. Albert B. Sabin, Cincinnati, Ohio. Recent advances in our knowledge of dengue and sandfly fever. *Am. J. Trop. Med. & Hyg.*, **4:** 198–208, 1955.

Twentieth. Harold Farnsworth Gray, Oroville, Cal. The confusing epidemiology of malaria in California. *Am. J. Trop. Med. & Hyg.*, **5:** 411–419, 1956.

Twenty-first. Dr. Leon H. Schmidt, Cincinnati, Ohio. The role of pharmacology in determining the rational use of therapeutic agents. *Am. J. Trop. Med. & Hyg.*, **6:** 212–223, 1957.

To this list, we are honored and privileged today to add the name of the 22nd Craig Lecturer, Dr. L. L. Williams, Jr., who will speak about "Malaria Eradication—Growth of the Concept and Its Application."

MALARIA ERADICATION—GROWTH OF THE CONCEPT AND ITS APPLICATION[1]

LOUIS L. WILLIAMS, JR.

Pan American Sanitary Bureau, Regional Office of the World Health Organization, Washington 6, D. C.

REMINISCENCE

The concept of the eradication of malaria was born in the United States but its growth was slow. It had to overcome the dictum that while malaria could be controlled eradication should not be attempted.

This was so in spite of the urging of one man who did have the vision of eradication and who stated it forcefully, Dr. Frederick L. Hoffman. Perhaps his vision was too broad for his day and the tools at hand too expensive or not sufficiently effective. Perhaps he ranked quinine too high as an antimalaria tool or else he described his plan too much as a movement and too little as a specific program. In any case, it received no support at the time.

In his paper, "A Plea for a National Committee on the Eradication of Malaria" (Hoffman, 1916), he told of the adoption by the Second Pan American Scientific Congress on 8 January, 1916, of his resolution "That all American countries inaugurate a well considered plan of malaria eradication and control based upon the recognition of the principles that the disease is preventable to a much larger degree than has thus far been achieved, and that the education of the public in the elementary facts of malaria is of the first order of importance to the countries concerned." He stated his belief that eventually the disease will be eliminated by economic progress, land improvement, drainage systems, etc., and proposed that a movement be organized to accelerate these factors and bring in extensive quinine treatment by physicians. To further this movement he proposed that there be organized a National Committee on the Study and Prevention of Malaria, and further stated that "The entire movement for malaria eradication, however, requires to be centralized in much the same manner as . . . the nation-wide efforts against infant mortality, tuberculosis, cancer, etc.," and that such a movement should be under the auspices of the American Medical Association.

In November of that same year he read a paper (Hoffman, 1917) entitled "A Plea and a Plan for the Eradication of Malaria." Unfortunately the published version of this paper was a summary barely sketching the details of the Plan, which included both medical and large engineering drainage and irrigation projects.

In spite of his great influence, (he was the father of the National Malaria Committee, the forerunner of one of our parent societies) his words carried no weight at the time, for control itself was hardly established and eradication did not then supplant control. This is not strange, for in the early days of public health practice in this country the two words were often used interchangeably. When the rank and file of public health workers referred to disease eradication they meant the elimination of an endemic disease as a public health problem, not as eradication *per se*. About the time of World War I there was a quiet but persistent drive on the part of semantic purists to eliminate the term "disease eradication" from the vocabulary of earnest public health workers unless it was followed by the qualifying phrase "as a major public health problem!"

Particularly was this true of malaria. The budding malariologists had this point of view persistently drilled into them by their elders who held that it was almost presumptuous to attempt eradication. Control the disease through antilarval works, yes; eliminate it, no—fantastic! Perhaps they were right, in the context of their time, for in those early days antimalaria work was a strictly local, urban undertaking and it was inconceivable that reinfection would not be certain and quick whenever maintenance of control ceased.

The general picture was not encouraging. Malaria was essentially a rural infection and its transmission was easy because of prolific anophe-

[1] The Twenty-second Annual Charles Franklin Craig Lecture delivered at the Annual Meeting of the American Society of Tropical Medicine and Hygiene held in Philadelphia, Pa., on October 30, 1957.

line production from innumerable water deposits dotting the countryside. Most dwellings were concentrated in small villages, yet there were many isolated, ill constructed houses scattered throughout the farmlands. The only attacks known were (a) by drainage, an impossibly expensive procedure for agricultural areas; (b) by weekly oiling which was almost as prohibitive in cost; and (c) by screening, an ineffective control method because the typical tenant shack, full of holes and cracks, gave easy ingress to the vector mosquito. Also, where malaria was rife, there were no local, rural health organizations to direct the work and assure its continuance.

There were County Boards of Health in the South, prescribed by law, but they were for the most part composed of unpaid selections from among the overworked local physicians who often did little more than post measles quarantine signs and hope for the best. Growth of rural health departments operating on a county wide basis did not really commence until the advent of extracantonment work in the deep South as we entered World War I.

The leaders in public health were city health officers often perturbed by adjacent high malaria rates but not jurisdictionally responsible for them. Antimalaria work was not a function of Government; indeed, even research in malaria control was not considered to be a responsibility of Government before 1912. And this was curious, because Government had engaged in control studies and works commencing with Gorgas in Havana in 1900 (Le Prince and Orenstein, 1916) and extending through the period of construction of the Panama Canal.

In 1912, Dr. Henry R. Carter, who had long worked with Gorgas, had come up from Panama and been stationed at the small Baltimore Marine Hospital, of which he was often the only staff officer. I was fortunate in being interned under him during the next year, when I learned from him the preparation and examination of the thick films later used in making malaria indexes.

Through his efforts the Public Health Service had developed a belated interest in malaria control and reluctantly permitted him to put some time into preparing an antimalaria educational program (Carter, 1914).

Meanwhile, Dr. Rudolph H. von Ezdorf, while serving with only one interne as Medical Officer in Charge at the Mobile Marine Hospital, was authorized, through Carter's urging, to commence malaria field studies and to originate the Office of Field Investigations of Malaria. This is the office which was later moved to New Orleans, transferred after the war to Memphis under Dr. Lunsford D. Fricks and, later, both under my command, to Richmond (1926) and to the National Institute of Health (1930). From this original office, von Ezdorf and his assistants, Drs. Robert Derivaux, T. D. Haas and Herbert Taylor, could study the prevalence and distribution of malaria in the field, and could begin the education of communities (Von Ezdorf, 1912; 1914a, b, c; 1915; 1916; Sparks, *et al.*, 1918). In this they had the invaluable help of Sanitary Engineer Joseph A. LePrince, himself just returned from antimalaria work under Gorgas and Carter in Panama. However, any investigation of methods of malaria control was entirely dependent on their ability to induce local authorities to finance locally the actual antimalaria work.

I know how hard their task was because in the Spring of 1914 von Ezdorf, an immune, was sent to Veracruz and Tampico to aid the Mexican Government in fighting yellow fever; Dr. Carter was sent to Mobile to take charge of the malaria office and the hospital; and my internship was cut short so that I could follow him there to take over the hospital. This was necessary because Dr. Carter and his small staff had to meet certain field commitments as the new malaria season opened, and the choice fell to me as the only available U. S. malariologist (so termed) who could not only take charge of the hospital but who also knew how to examine the thick films to be sent in by the field force. As the films came in at the rate of 75 a day, no time hung heavy on my hands.

There were other difficulties. Dr. Carter had by then become sure in his own mind that impounded waters were the key to malaria in the Southeastern States. Power companies were multiplying impoundments, malaria became epidemic around the new ponds, and resulting law suits threatened some companies with bankruptcy. Not knowing how to prevent anopheline production in the lakes, they appealed to the Government for assistance.

The Bureau of the Public Health Service at that time held the view that the problem was an industrial one, for Government was in a trust-busting mood and certain practices of big busi-

ness were frowned upon. Dr. Carter was refused authority to spend Federal funds even on travel on such a venture. He solved the immediate problem by taking annual leave and permitting the power companies of North and South Carolina to pay his expenses (he refused any fee) and some of his earliest impounded water studies were made in this way.

Later, through persistent education, he overcame objections of Headquarters and with LePrince and T. H. D. Griffitts, expanded studies of impounded waters (Carter, 1914, 1915; Le Prince, 1915).

Those were days of struggle, of education, of slow introduction of the concept of malaria control. This was true particularly of education, for it quickly became apparent to Carter that very few antimalaria works were likely to be approved at local expense unless the people knew what it was all about and would support local authorities who must spend the taxpayer's money. It was pioneer work in an exciting period, but it was hardly conducive to thoughts of eradication.

A word more may be said about education, as it was of the greatest importance for what was to follow. Dr. Carter, in 1914, produced The Malaria Catechism which was issued under the descriptive title of "Malaria, Lessons on Its Cause and Prevention, For Use in Schools." This pamphlet, destined to become the Government Printing Office's "Best Seller," was of the question and answer type and had the rare quality of leading the reader to the end. Issued by the hundred-thousand in numerous printings, reprinted by many State Health Departments in even greater quantity, and placed in most Southern schools off-and-on for three decades, it educated a generation and prepared it to understand and support wholesale antimalaria work and, eventually, to accept the concept of malaria eradication.

EARLY HISTORY OF MALARIA CONTROL

Urban at first, malaria control had survived the purse-chilling advertisement of its great cost during construction of the Panama Canal, and eventually extended into rural areas, the home of malaria. But progress was slow and was dependent upon development of methods cheaper than drainage. Directors of control operations did not then think in terms of eradication, for the hazard of reinfection from adjacent rural malarious areas was very real.

Von Ezdorf, active in the field, succeeded in initiating a few small malaria-control programs in 1912 and 1913. Later, Carter and LePrince (the greatest of teachers), noting the close association of malaria with impounded waters, devised fluctuation of water levels as a means of providing acceptable control of anopheline production (Carter, *et al.*, 1916) at hydroelectric ponds, and thus drew industry into the picture. Improvements in methodology slowly followed, finally culminating in the brilliant developments of Dr. Eugene Bishop and his staff who made possible the healthy development of the Tennessee River Valley (U.S.P.H.S., 1947).

In 1916 the International Health Division (now the Rockefeller Foundation) entered the field with personnel and with "matching funds," and control of malaria in and about small towns expanded rapidly; for these matching funds made it much easier to induce officials to appropriate money for drainage and oil spraying.

This was before the days of "species sanitation." An anopheline was an anopheline, and breeding places of all species were suspect and subject to attack. Ponds and swamps were drained, ditches straightened and streams rechanneled or sprayed weekly with kerosene. No thought went beyond simple malaria control through antilarval works which rarely extended for more than a mile from population centers. Such works did halt urban transmission; but a total absence of malaria was not dreamed of as it was obvious that rural malaria would always be a reinfecting hazard. Some control was practiced in some populous rural areas but any reasonable degree of malaria reduction was termed malaria control and lowering of rates from categories of 25 per cent or 40 per cent to 10 per cent or 5 per cent was acceptable.

This extension into nearby rural areas accelerated as cheaper oil larvicides were discovered, and wind-blown Paris green dust further extended them. Introduced by Barber and Hayne (1921), Paris green was first used as a 1 per cent road-dust mixture thrown by hand. Later it was blown from hand-operated cotton dusters as a 10 per cent mixture in lime or talc, then from power blowers as developed by LePrince and Johnson (1929) and, finally, from airplanes by King and Bradley (1926) and by William and Cook (1927) and Cook and Williams (1928).

Mechanical procedures became better and better and costs were correspondingly lessened.

But research lagged until the Rockefeller Foundation set up a field station for Dr. Samuel T. Darling at Leesburg, Georgia; and later another for Dr. Mark F. Boyd at Edenton, N. C., subsequently moved to Tallahassee, Fla. These two investigators not only gave a healthy impetus to research, both laboratory and field, but also trained a large number of enthusiastic young men from home and abroad. Many of these, Fred L. Soper, Paul F. Russell, W. G. Smiley and Arnoldo Gabaldón to name only a few, were destined, themselves, to train many more malariologists as well as to make major contributions in malariology and to play a significant role in the then distant development and practice of eradication.

At the same time LePrince, and also Coogle (1927), were devising a method of actually mosquito-proofing warped and porous farm tenant homes, even those of poorest construction. So easily and so cheaply could this be done, at an average cost of only ten dollars per house, and so popular and effective was the method that a number of counties in the cotton section adopted mosquito-proofing as a regular function of the local health department (Fullerton, 1929).

All of these methods and the growth of rural county health departments during the nineteen twenties led to successful county-wide malaria control programs in a number of states.

Freedom from malaria was achieved, however, by repeated dusting and constant upkeep of screens accompanied by the use of household sprays until diversified farming, with its improved diet, better housing and advancing industrialization led to such an improvement in the economic scale and habits of life that many of these counties, and even some states, became rid of the disease.

Curiously enough, the depression of the thirties did not change the general downward trend of malaria in the Southern United States. In fact, it was a boon, for relief labor became available for antimalaria drainage and over 60,000 miles of ditch were dug in the most malarious states. This went a long way toward breaking the back of the remaining malaria problem (Williams, 1945).

Soon thereafter war-time antimalaria works greatly expanded the program. Commencing in 1942 vigorous malaria control programs were initiated inside all the Army training camps in the South. Simultaneously, the Public Health Service, through my Office of Malaria Control in War Areas, and in cooperation with state and county health departments, extended them to the environs of all nearby towns. War industries and their adjacent villages were added and soon 2,200 antimalaria programs were under way in the 20 states in which malaria was still a hazard. Before the end of World War II the total amount of malaria in the country was very small. The average of all thick film indexes was less than 0.5 per cent and none exceeded 10 per cent (U.S.P.H.S., 1943).

In view of this small residuum of malaria and with the knowledge that military demobilization would introduce a large number of carriers into the civil population, I proposed to our Bureau in 1943 that the Public Health Service sponsor and coordinate a program for malaria eradication to be executed through state and local health departments, and that the Congress be requested to make an appropriation, in addition to that for war work, with which to expand existing programs into all parts of participating counties and to extend them to include all other counties in which malaria was still present.

Belief in the success of such a program was justified because (1) endemic malaria had disappeared from the most temperate portions of the country to reappear infrequently as small, sharply-circumscribed, short-lived epidemics, and those only where some temporary local conditions caused production of an excessive density of a vector species (Williams, 1945); (2) years of persistent control in every malarious state followed by the extensive work of the relief agencies had prevented the expected cyclic rise of 1940 or 41 (Williams, 1941); (3) the far-flung work of the war period, at that time entering the maintenance phase, had made malaria hard to find in hitherto large endemic areas. It was apparent that intensification of effort in existing control areas and extension into all other, and not too large, endemic areas would surely eradicate the disease from the entire country. Moreover, the exigencies of the war had conditioned the Congress to appropriate funds for antimalaria works.

Besides, the technique of eradication had been demonstrated. Soper, in 1938 (Soper *et al.*, 1943), while trying to reduce further the *Aëdes aegypti* index in Brazil (then at, or below, one) had progressively intensified the supervision at

all of his control cities and towns, had inaugurated adult capture teams, had introduced effective methods of checking on all phases of the work, and had achieved full coverage. Also, he had achieved eradication. When he used those methods in Northeast Brazil from 1939 to 1941 (Soper, *et al.*, 1943) he repeated this success and eradicated *Anopheles gambiae*. These campaigns demonstrated that no new professional methods were needed, that the technic of eradication was solely an administrative one. It can be summed up in the expression "check, cross-check and recheck."

In 1943 the concept of eradication was accepted, but its terminology was not. Too many years had been spent in emphasizing control in the belief that eradication was unjustified because of its cost and because of the certainty of reinfection. In 1945 an appropriation for malaria eradication was requested; but due to traditional opposition to the term "eradication" it was secured in the name of "The Extended Malaria Control Program," (Williams, 1945). Not until 1947 were funds appropriated unequivocally for malaria eradication to be carried out through a campaign designed to run over a period of 5 to 7 years. However, DDT became available as the work commenced, and by 1950 the campaign was successful.

I will not recount the history of that campaign under Dr. R. A. Vonderlehr, under Sanitary Engineer Mark D. Hollis and under Dr. Justin Andrews. Its memory is still vivid in your minds and it has been well described by Andrews and his coworkers (1954). Besides, many here present today actively participated in its accomplishment. Nor will I describe the brilliant studies and control work in California by Drs. W. T. Hermes and Stanley Freeborn and Sanitary Engineer Harold Gray whose fundamental studies made possible extension of eradication to the Far West. However, the early history of the movement has been here recounted in some detail because it shows that malaria eradication would have been successful by the old-time methods of screening and of attacking the mosquito in its breeding place, even though it would have required painstaking consolidation of gains after each successive step in a long, expensive, up-hill fight. These pauses and these steps are no longer necessary, for residual insecticides ignore both the size and number of breeding places and costs are now calculated solely on the number of houses and square footage of their walls. But, failing the new, old methods could still be resorted to.

DDT

And now, a brief word about DDT which, through ease of application and cheapness, makes malaria eradication possible on a global basis. Although it had been extensively used overseas by the Military, DDT first became available for malaria eradication in the United States in 1946. It took advantage of the ecology of the anophelines, nearly all of which are active only from dusk to dawn. Most species rest after the flight from the breeding area or daytime roost, to the feeding site. Anyone who has watched the loggy, staggered flight of a recently engorged female mosquito can well understand her postprandial predilection for the nearest resting place, the bedroom wall. And this routine will be repeated from three to five times during the 2 weeks required for the extrinsic incubation of malaria. Therefore, before transmitting the disease each anopheline feeding on man must not only endure the vicissitudes confronting so fragile an insect, but she must also resist the normal urge to alight on an accustomed surface, now become deadly, and resist this urge from eight to a dozen times! This she does not do.

The introduction of residual insecticides profoundly affected not only antimalaria methods but antimalaria thinking. Sprayed but once or twice a year on inner walls of houses it kills, by contact, the roosting anophelines and continues to do so for months. It is a selective method aimed only at house-frequenting, individual mosquitoes. When it is sprayed on all inner walls of every house in a malarious area, malaria transmission is terminated.

No longer need attention be given to drainage or larviciding with their great cost, nor to any specific anopheline species; rather is attention focussed solely on those individual insects which are, or may become, infective. At last the parasitic *Plasmodium*, the agent of infection, is under attack outside of the human body and "anophelism without malaria" is the aim.

Although easy to apply and popular because of the incidental freedom from annoying insects, the significance of DDT lies in its low cost, effectiveness, and ease of application. Just as progressive reductions in antimalaria costs always encouraged expansion of control programs, DDT

inevitably led to eradication campaigns. Fortunately this is not the only anopheline adulticide, for resistance to it has developed in a few species in a few areas and may do so in others, forcing insecticide substitution. Resistance also highlights the need for acceleration of programs to achieve eradication before anopheline resistance can develop too greatly. So far it has not halted a single campaign.

DRUGS

There is another string to the malariologist's bow: chemotherapy. Had residual insecticides not been discovered, the parasite could be attacked, and this time within the human body, with one or all of the three new, synthetic, antimalarial drugs.

Many thousands of compounds were screened in lower animals for antimalarial activity by Dr. G. Robert Coatney and his coworkers (1953). The few compounds showing activity were tested in human volunteers and this resulted in the discovery of several drugs of higher antimalarial activity than those previously used; viz., chloroquine and amodiaquine of the 4-aminoquinolines, and primaquine of the 8-aminoquinolines. Later, Dr. Hitchings (Falco, *et al.*, 1955) found that pyrimethamine, a compound belonging to another group, also had unusual antimalarial properties.

Chloroquine (or amodiaquine) cures acute attacks due to any of the species of *Plasmodium* and radically cures *falciparum* malaria. Primaquine radically cures *vivax* infections, while pyrimethamine, the best suppressive drug, has been shown by Drs. Martin D. Young and R. W. Burgess (1957) to have the remarkable power, when ingested by a malaria carrier, of preventing development of sporozoites in the mosquito and thereby terminating transmission. This effect persists for a number of days.

In any case, when transmission is interrupted for 3 consecutive years malaria dies out of the population, for *falciparum* malaria habitually persists in an individual for only 1 year and *vivax* for only 2 to 2.5. Some quartan may persist longer but, whenever transmission ceases, it also dies out. Upon cessation of residual spraying at the center of operations, the program must continually expand at the periphery to prevent reinfection from neighboring areas. Also, for a period, surveillance teams must continue vigilant observations to detect and stamp out missed foci, or reinfections of foreign origin. Gradually, health departments assume this function as a normal part of their epidemiological work, but discontinuance of surveillance must await global eradication.

Malaria-eradication campaigns might be shortened and costs reduced by abandoning the practice of continuing house spraying during the second and third year after the first year of full coverage. When malaria transmission has been halted the major drop in the parasite rate occurs during the first year; usually a reduction of about 90 per cent. During the subsequent 2 years surveillance more intensive than usual might substitute for nation-wide spraying.

Under these circumstances, when a surveillance team discovered a focus of infection, residual sprays would be used according to accepted practice but, in addition, chemotherapy should be introduced promptly. Administration of drugs is here important because a focus of infection after only 1 year of spraying is likely to consist of more than a single case, perhaps scattered cases throughout a community, and the malaria parasites should be rendered noninfective to the mosquito before a local epidemic can occur and before any of these infected people visit distant places. Speed is essential and drugs can be administered quickly. Active cases can thus be cured promptly and, through the use of pyrimethamine, the gametocytes of infected individuals can be rendered harmless.

This drug, or other therapeutic agent, can also meet the need of the eradicationist under special situations where residual spraying is without effect. Where houses are without walls, where malaria-carrying mosquitoes bite outdoors during either the day or night, or where people are nomadic, pyrimethamine or chloroquine (or amodiaquine) could be combined with all *ad lib.*, salt. If this is not feasible because people drink from "salt wells" or get unmedicated salt from other sources, peripatetic drug teams could periodically administer a maximum dose of pyrimethamine to each individual suspected of infection. Although the maximum-dosage interval is yet to be determined, it is known that pyrimethamine (or chloroquine) can be given in salt daily over long periods of time without evidence of toxicity and that this procedure has worked well under both controlled and field conditions (Pinotti, *et al.*, 1956).

So much for the scientific tools which are ade-

quate if properly used. However, the definitive technique of eradication is administrative. Firm, yet flexible enough to adapt to the emergencies, changed conditions and frustrations so familiar to antimalaria workers, the administration must provide full supervision of each activity and must devise checks, cross-checks and rechecks so well integrated into the total program as to assure that no essential part of a house goes unsprayed and no house is overlooked.

INTERNATIONAL ASPECTS

As programs reach national boundaries stimulation of peripheral expansion and coordination of intergovernmental or regional programs becomes a function of international agencies, namely the Pan American Sanitary Organization and the World Health Organization (WHO), working through its Regional Offices. These organizations, themselves, do not carry on direct operations. Antimalaria programs are prosecuted in each country by the government of that country largely through its own resources and utilizing its own nationals. Upon request of government, the WHO or the Pan American Sanitary Bureau (PASB) supplies technical experts who help assess the national problem, plan operations, outline administration, train opposite numbers and remain as consultants only so long as may be needful. These organizations also provide fellowships to nationals for training abroad in malariology and the administrative techniques of eradication. This is wholly in keeping with the basic principle of helping governments to build strong, national health administrations. Also, it assures a competent local staff to carry on surveillance after insecticidal work terminates.

Malaria eradication as an integrated international health program was first proposed by Dr. Soper in 1950 to the XIII Pan American Sanitary Conference of the PASB which adopted hemispheric malaria-eradication as its objective. Insufficient funds prevented an organized drive for concerted action and the only progress during the next 4 years consisted of some expansion of existing control programs. In 1954 the XIV Conference gave the Bureau a mandate to stimulate national eradication campaigns, coordinate them and speed the eradication of malaria before anopheline resistance to insecticides could develop. Also, it appropriated some money and Dr. C. A. Alvarado, the distinguished Argentinean malariologist, organized within the Bureau the present Office of Malaria Eradication.

The following year (1955), the World Health Assembly, by resolution, took similar action and the world-wide eradication of malaria became the ultimate objective of the WHO.

Upon its inception the WHO had accepted certain UNRRA malaria-control programs bequeathed to its Interim Commission in 1946. Immediately, malaria control was placed in the top priority group and efforts were made to induce governments to initiate or expand national malaria-control programs in all member countries in which the disease was a serious problem. Nine years later, the restricted idea of control was abandoned and the concept of eradication took its place.

By the end of 1956, largely due to the work of the WHO and the PASB, the disease had been eradicated from five countries and five territories and islands, and antimalaria programs were in operation in 94 others. Of these, 45 have already converted long-established control programs into eradication campaigns; the other 49 continue control programs.

In all probability the majority in this last group will soon convert to eradication, for there is a definite governmental trend away from control, with its annually recurring expenditures, and toward eradication which permits safe conversion of control appropriations to other public health programs. This trend is manifest also in the thinking of the governing bodies of international agencies. The United Nations International Children's Emergency Fund (UNICEF) has recently multiplied the quantity of supplies furnished to antimalaria programs of governments; adding new countries to those already receiving assistance only if the program is one for eradication. Authorities concerned with the U. N. Technical Assistance Program similarly recommend that cooperative control programs be converted to nation-wide eradication campaigns lest eventual withdrawal of outside support result in return of epidemic malaria. In addition, the United States bilateral program, the International Cooperation Administration, is pressing for conversion of control into eradication in all of the many countries in which it cooperates in anti-malaria works.

The international health agencies also seek sources of material assistance for nations unable

themselves to secure needed supplies. One such source is the technical assistance program of the United Nations, another is UNICEF. Sometimes all cooperate in a single program with funds from WHO and Technical Assistance providing professional personnel; UNICEF supplying insecticides and equipment; and Government directing the program and supplying the labor. The international funds in these programs are often in the proportion of two thirds from UNICEF, one fourth from Technical Assistance and only one twelfth from the international health organizations.

Response to the numerous calls from governments for expert consultation in the whole field of Public Health strains the limited resources of the PASB and the WHO and leaves insufficient funds available in regular budgets for malaria eradication activities. Therefore, each agency, in resolving upon continental and global eradication, respectively, has made provision for special funds for malaria eradication.

The WHO Special Fund is as yet of restricted[2] usefulness as it consists only of soft currency or services in kind. The special fund of the PASB receives $100,000 annually from the Regular Budget for its Office of Malaria Eradication. From government sources it has already received, in hard currency, 1.5 million dollars from the United States, 0.5 million ($100,000 annually for 5 years) from the Dominican Republic and 0.33 million from Venezuela. These funds will be used principally to: (1) recruit international consultants in number sufficient to fill the immediate needs of governments in the region of the Americas; (2) to supplement existing training facilities in Spanish and English to meet all the training needs in the hemisphere; (3) to develop evaluation surveys; and (4) to supply materials not locally procurable for which no other source is available.

As a result all countries and territories of the Western Hemisphere are actively at work or have plans for the elimination of malaria, which has been eradicated from the United States and Chile and from four Caribbean islands. It is anticipated that within the next 2 years programs will be under way in all countries of the Americas.

In the European region and Eastern Mediterranean region malaria has been eradicated from two countries and from Sardinia and the Gaza Strip. Eradication campaigns are in progress in twelve others and are planned for five more.

In the South East Asia region and the Western Pacific region campaigns for eradication are under way in seven countries and are planned for seven more.

Nothing specific can be said at this time of antimalaria activities in Communist China, but those familiar with her malarious areas perceive no technical difficulties standing in the way. Transportation difficulties are obvious in the malarious southwest portion, where the terrain is rugged. However, roads are not entirely impassable, and the local mosquito vector of malaria is notoriously susceptible to DDT.

Although malaria has been eradicated from Reunion and Mauritius and campaigns are progressing in Madagascar and are planned for four others, nothing is as yet visualized in the 29 which harbour the great problem of the African region lying south of Sahara and north of Southern Rhodesia. This is the one area in which serious doubts have been expressed of the feasibility of malaria eradication. The Continent's enormous size, great distances and lack of roads coupled with the depressed economy of the millions of people at risk make eradication seem an Utopian goal. But this need not be so, for the obstacles are chiefly administrative. DDT does tend to adsorb on some mud walls and *A. gambiae* in northern Nigeria has become resistant to dieldrin, the newest residual insecticide. On the other hand, pyrimethamine has twice proven itself to be effective against African malaria, though the optimum frequency of dosage and method of administration remain to be determined.

True, no country in this region has yet essayed the eradication of malaria but 15 of them have embarked on control programs of varying size and duration, any one of which may at any time set an example by converting, for there is no place in the world from which malaria cannot be eradicated.

CONCLUSION

Large as it is, the global program for eradication of malaria is not beyond the economy of nations. Estimates of annual expenditures vary from 45 cents per capita where labor cost is high to only 11 cents where it is low. As the global campaign approaches its end, inevitably the residuum of malaria will be found only in coun-

[2] Since presentation of this paper the U. S. Government has given 5 million dollars to to this fund in WHO and 2 million more to PASB.

tries with low economic resources to which the cost of eradication may seem prohibitive. It is just as inevitable that more assistance will flow from the more fortunate nations, for the hazard of their reinfection will persist and their own resources will be greater. When that time comes there should be no difficulty in achieving the ultimate objective of global malaria eradication.

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AN OUTBREAK OF INFECTIOUS HEPATITIS ON A COLLEGE CAMPUS

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INTRODUCTION

Infectious hepatitis has become more frequently recognized as a seasonal epidemic disease, but it has been uncommon to observe outbreaks deriving from a common source of infection. Five outbreaks have been attributed to contamination of common water supplies (Gauld, 1946; Gunnar, 1947; Neefe and Stokes, 1945; Tucker, Owen and Farrell, 1954; Nat'l. Off. Vital Stat., 1956; Reed, Bancroft, Doull and Parker, 1946) and it is believed that smaller outbreaks have had their origins in contaminated food (Murphy, Petrie and Work, 1946) or milk (Peczenik, Duttweiler and Moser, 1956).

In the fall of 1952, a sizable epidemic of infectious hepatitis was observed among the faculty and student body of a privately-operated coeducational college in South Carolina. At least 222 individuals were affected, the over-all attack rate being 8 per cent. The outbreak was abrupt in onset, short in duration, and all of its characteristics suggested a common source of infection. Although the college dining room was definitely implicated, intensive study revealed neither the source nor the vehicle of infection.

The outbreak described in this paper is reported primarily because it exhibited some interesting and unusual epidemiological characteristics. It will be shown that over 2,100 persons were exposed in an apparently random manner. Thus an unusual opportunity was presented for a study of the influence on susceptibility of such variables as age, rural or urban residence, number of siblings, history of jaundice, and veteran status. These variables did not seem to influence the attack rates among the groups at risk.

The outbreak also is unusual because it began with a single recognized "pilot" case, was followed by a sharp, single wave of cases, and an apparent absence of secondary cases. This lack of secondary cases possibly was due to the extensive use of gamma globulin.

Locale. The college is located on the outskirts of a city of 70,000 in northwestern South Carolina. It had been housed in a new physical plant for 7 years prior to the outbreak. The enrollment of the school was 2,572 in 1952, including 2,112 resident students and 460 nonresidents. The faculty and staff comprise an additional 214 persons.

The resident students live in dormitories on the campus. All but a few of the faculty and staff also live either in the dormitories or in separate family housing on the campus. The dormitories vary in capacity from 30 to 300; all were overcrowded in 1952 with four or five students living in rooms designed for two or three.

All resident students eat in a common dining hall where seats are assigned and attendance is mandatory. For the most part, the resident faculty and staff also eat in the dining hall, but their attendance is not so closely supervised.

Other places of social contact are the classrooms and daily chapel, where attendance is mandatory; the library; "snack-shop"; student union; and the dormitories.

Although students are not closely restricted to the campus, their time is well filled with academic and on-campus social activities, and there is relatively little social intercourse with the nearby community or with other colleges in the vicinity. There are no fraternities or sororities in the usual sense, but each member of the student body is enrolled in one of 32 literary societies. These societies form social nuclei and are individually represented in athletics, dramatics, and other campus activities.

The college has a modern infirmary of 20 beds that is staffed by resident nurses and attended by designated physicians of the nearby city.

Recognition of the outbreak. In mid-October of 1952, approximately 6 weeks after the beginning

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of the fall term, a slight increase over the normal rate of infirmary admissions was noted. Most of the illnesses were mild, and many were described as upper-respiratory infections. Some of the patients at this time were later readmitted with jaundice. By the end of the month, it became apparent that an epidemic of major proportions was occurring.

The college requested assistance of the State Board of Health which in turn requested aid of the U. S. Public Health Service. Between November 6 and 11 the Communicable Disease Center (CDC) medical investigators had interviewed and examined each member of the student body, faculty, and staff. Individuals with apparent illness were hospitalized in the infirmary or in facilities improvised in two dormitories.

METHODS OF STUDY

Criteria for diagnostic classification. More than 300 persons were ill during October and November. Of these, 281 had symptoms or signs suggestive of infectious hepatitis. For purposes of this study, this group was subdivided as follows:

1. *Hepatitis with icterus.* One hundred and eighty persons were clinically icteric, and had physical findings compatible with a diagnosis of infectious hepatitis.

2. *Hepatitis without icterus.* Forty-two individuals, never icteric, had hepatic tenderness on palpation or, in a few instances, on percussion. In addition, each had one or more additional signs or symptoms compatible with a diagnosis of infectious hepatitis.

3. *Doubtful hepatitis.* Fifty-nine individuals were characterized by a symptomatology like that in Group 2, but hepatic tenderness was not demonstrated.

The 222 persons in the first two categories alone, hepatitis with icterus, and hepatitis without icterus, are the basis for most of the case data which follow.

Demographic information. Data concerning age, college residence, grade or class, society membership, dates of absence from college, seating arrangements in the dining hall, menus, and infirmary visits and admissions were obtained from the college administrative records.

A mimeographed questionnaire, which asked for information regarding eating habits, life residency, size of family, history of infectious hepatitis, and other pertinent variables, was circulated among the entire university population about a month after the beginning of the epidemic. There were 2,634 questionnaires returned, representing 95 per cent of the school's total population.

Environmental information. Water, milk, and food supply operations and records were studied by engineers of the State Board of Health. Operating records for the water supply, including those of repair, pressures, and chemical and bacteriologic analyses were obtained from the city authorities. Records of on-campus repairs, system layout, pressure fluctuations, and the like were provided by the college maintenance crew.

Prevalence of infectious hepatitis in the community. Information was gathered from official records, from school reports of absenteeism, from personal interviews with school nurses, health department personnel, and selected physicians. Histories were obtained from 3,400 students of three high schools in the community, and reported illnesses were individually evaluated for evidence of hepatitis. Infirmary records of two other smaller colleges in the area were reviewed, and ill students were interviewed and examined.

RESULTS

Clinical features. In general, the illnesses observed were mild and the acute phase was of short duration. Onset was most frequently described as gradual, but a reasonably precise dating of first symptoms was possible.

Table 1 gives the frequency with which certain symptoms or signs were noted in the 180 patients with icterus, and in the 42 whose illness was classified as hepatitis without icterus. Headache and nausea were noted by three-fourths of the patients in each category. Diarrhea, constipation, and abdominal discomfort were equally frequent in each group. Dark urine and light stools were much more frequently reported in the icteric group, as might be expected. Hepatic tenderness was noted in 70 per cent of the icteric group, and (by definition) in all of the nonicteric category. Hepatic enlargement was observed in one-half of the icteric cases and in one-third of the anicteric group.

Not shown in Table 1 is the frequency of various symptoms and signs in the group designated as "doubtful hepatitis". Except for hepatic enlargement and tenderness (the latter absent

by definition in the "doubtful" group) this group presented a frequency of symptoms and signs almost identical to the "hepatitis without icterus" group. This suggests that the "doubtful" might actually be included among those diagnosed as hepatitis; however, the doubtful group has been excluded from further consideration in order to keep the diagnostic categories as sharp as possible.

TABLE 1

Clinical findings in 222 individuals considered to have infectious hepatitis, 1952

Symptom or finding	Icteric hepatitis (180 patients)		Anicteric hepatitis (42 Patients)	
	Number	Per cent	Number	Per cent
Fever (symptom)	136	75.6	13	31.0
Headache	126	70.0	32	76.2
Anorexia	135	75.0	21	50.0
Nausea	142	78.9	32	76.2
Vomiting	105	58.3	12	28.6
Diarrhea	40	22.2	9	21.4
Constipation	67	37.2	17	40.5
Dark urine	134	74.4	14	33.3
Light stools	53	29.4	4	9.5
Abdominal discomfort	100	55.6	25	59.5
Enlarged liver	91	50.6	14	33.3
Tender liver	128	71.1	42	(100.0)

The course of illness was generally uneventful and, for the majority of patients, there was complete freedom from symptoms or objective findings in 2 or 3 weeks. In about 15 per cent of those ill, however, hepatic tenderness and enlargement, fatigue, and anorexia required extended periods of care, with several still convalescent 4 months after the onset of illness.

Chronologic occurrence of cases. The chronology of the 222 cases classified as hepatitis is shown in Figure 1 and Table 2.

The first case had its onset on October 4; one case occurred also in each of the 2-day intervals, October 14 and 15, 16 and 17, and 18 and 19. A sharp upswing in the number of cases began on October 24 and 25, when 13 onsets were recorded. The epidemic curve rose sharply until October 28 and 29, when 33 persons became ill. An additional 31 persons became ill on October 30

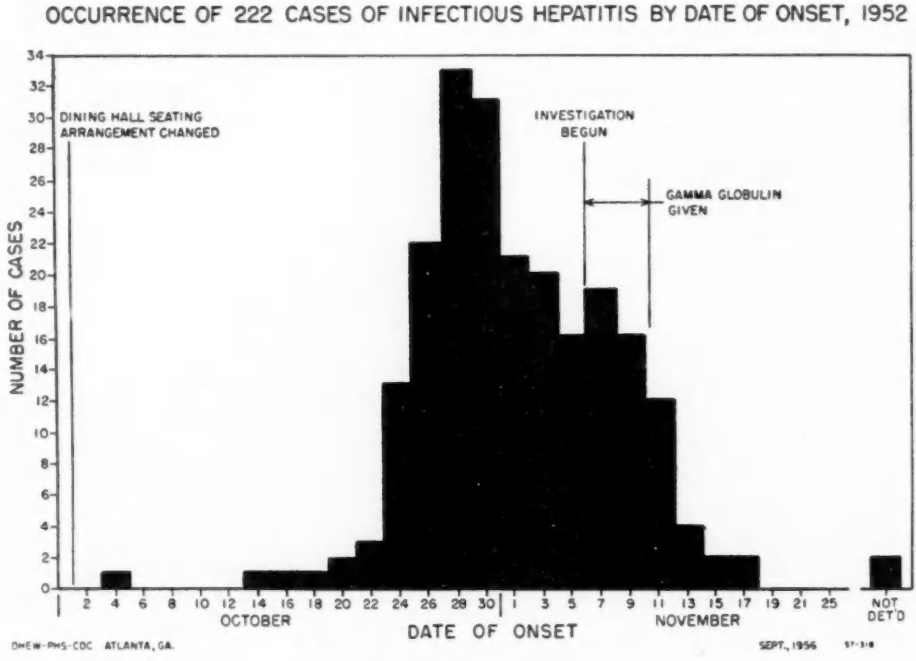

FIG. 1

TABLE 2

Chronologic occurrence of 222 cases of infectious hepatitis, by date of onset, 1952

Date of onset of symptoms	Number of cases	Date of onset of symptoms	Number of cases
October 2–3		November 1–2	21
4–5	1	3–4	20
6–7		5–6	16
8–9		7–8	19
10–11		9–10	16
12–13		11–12	12
14–15	1	13–14	4
16–17	1	15–16	2
18–19	1	17–18	2
20–21	2	19–20	
22–23	3	21–22	
24–25	13	23–24	
26–27	22		
28–29	33	Not determined	2
30–31	31		
		All cases	222

TABLE 3

Population, cases, and attack rates for infectious hepatitis, 1952

Group	Population	Cases	Attack rate (%)
Resident students	2,112	199	9.4
Nonresident students	460	2	0.4
Faculty and staff	214	21	9.8
All groups	2,786	222	8.0

and 31; following this date, the frequency of new cases declined gradually until November 17, when the last onset was recorded.

Except for the first case, and two for which the date of onset could not be judged precisely, all illnesses began in the 36 days between October 14 and November 17. Identical with the commonly described maximum incubation period of 35 days for infectious hepatitis, this span of time during which cases occurred is strong evidence that a single, short exposure to a common source of infection was responsible for the outbreak.

Use of gamma globulin. Between November 6 and 13, 2,704 persons on the college campus, including students, faculty, staff, and their families, were given gamma globulin in amounts ranging upward from 2 ml each. No cases occurred in individuals more than 10 days after they had received gamma globulin.

Distribution of cases according to resident status, and according to faculty-student status. The distribution of cases among resident and nonresident students, and among the faculty and staff, is shown in Table 3. Some striking features are apparent. Attack rates among resident students and faculty and staff members are identical. Only two of 460 students living off-campus were ill, however, giving an attack rate of 0.4 per cent. The attack rate in resident students and faculty was more than 22 times as high.

Clearly, the resident students and faculty were at some risk not shared by nonresident students. Aside from nonresidence in dormitories, little could be found to set apart the activities of nonresidents from residents, with one exception. Only 12 of the 460 nonresidents regularly or even occasionally ate in the dining hall. The two cases observed in the group were among the 12 who did sometimes use the dining hall, giving a "corrected" attack rate of about 16 per cent for nonresidents eating on campus.

Distribution of cases by dormitory residence. Table 4 gives the distribution of the 199 cases among resident students according to dormitory. All of the large dormitories, with 300 to 500 residents, exhibited remarkably similar attack rates, ranging from 8 to 11 per cent. The small dormitory "H", with only 30 residents, had a slightly higher attack rate of about 17 per cent.

In Figure 2 is shown the chronological distribution of cases, for students according to dormitory residence, for faculty and staff, and for students who lived at home. The chronologic occurrence of cases in these residence categories is remarkably uniform.

There is no evidence that the outbreak was localized to one or more dormitories. The data suggest a source of infection common to all who lived on the campus.

Distribution of cases by academic class or grade. Table 5 shows the frequency of attack in each class of the college and each grade of the small grade-school academy maintained by the college. Despite rather small numbers of students in the lower grades, attack rates are again rather uniform at all levels of instruction. There is no suggestion of localization of the outbreak to any

unit of population formed by school grade or college class.

Distribution of cases among literary societies. Among the 32 literary societies with one exception, attack rates ranged from 3 to 14 per cent. The one exception was a group of 80 students that had only one ill member. Because of the relatively small numbers in each group, none of the attack rates is significantly different from the others. Again, no localization can be demonstrated.

TABLE 4

Student population, cases and attack rates for infectious hepatitis in dormitories, 1952

Dormitory	Population of dormitory	Cases	Attack rate* (%)
"G" (men)	397	44	11.1
"Sm" (men)	395	43	10.9
"Su" (women)	495	41	8.3
"M" (women)	495	40	8.1
"H" (women)	30	5	16.7
"J" (men and women)	300	26	8.7
All resident students	2,112	199	9.4

* χ^2 (5 d.f.) = 7.17, $0.30 > P > 0.20$.

Distribution of cases in the dining hall. Early in the outbreak, it was observed that there seemed to be an unusual frequency of disease among students working in the dining hall. Before discussing this apparent localization of the epidemic, a few remarks about the dining hall may be worthwhile.

All regularly prepared meals, except the one on Sunday night, are served in the hall. The Sunday night meal, usually cold sandwiches and fruit, is prepared in the dining hall kitchen and distributed as a "box lunch" to the dormitories.

In the hall each meal is served in two shifts of about 30 minutes each. The hall is filled to capacity on the first shift, but is only about two-thirds full on the second shift. First-shift diners are college students, faculty, and staff. The second shift includes some students and faculty,

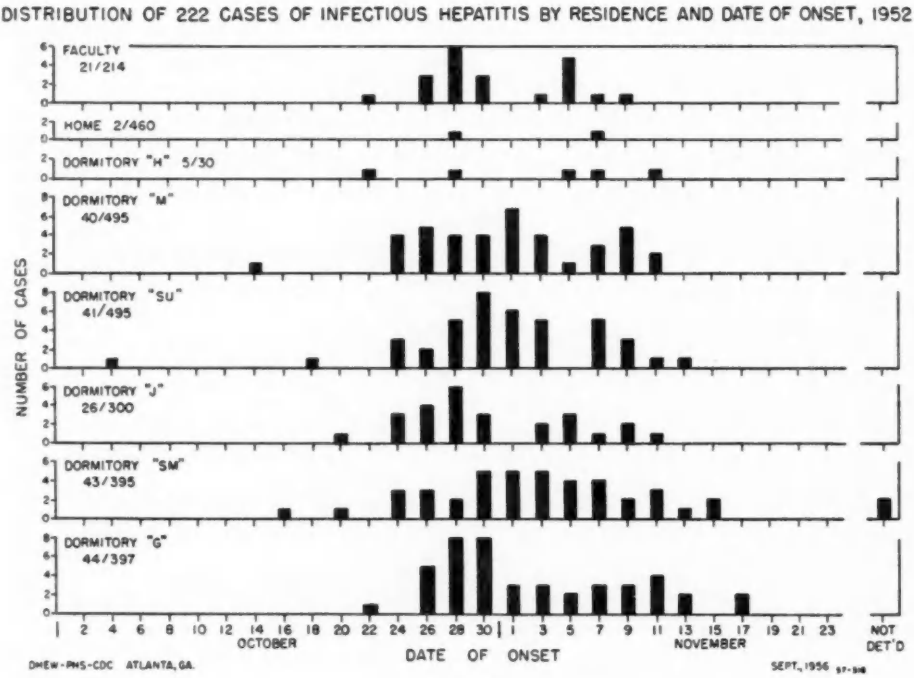
DISTRIBUTION OF 222 CASES OF INFECTIOUS HEPATITIS BY RESIDENCE AND DATE OF ONSET, 1952
FACULTY 21/214
HOME 2/460
DORMITORY "H" 5/30
DORMITORY "M" 40/495
DORMITORY "SU" 41/495
DORMITORY "J" 26/300
DORMITORY "SM" 43/395
DORMITORY "G" 44/397
NUMBER OF CASES
1 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 1 3 5 7 9 11 13 15 17 19 21 23 NOT DET'D
OCTOBER
NOVEMBER
DATE OF ONSET
DHEW-PHS-CDC ATLANTA, GA.
SEPT., 1956

FIG. 2

but it also includes the entire enrollment of the academy grades, and the children and wives of the faculty and staff. Despite these differences in composition, attack rates were nearly identical for each shift.

In the hall, diners are arranged eight to a table, with a definite and mandatory seating schedule. Usually, the schedule is changed by lot at about 3-week intervals. The seating arrangement at the beginning of the term on September 3 was changed on September 29. The second arrangement continued throughout the epidemic and well beyond the normal 3 weeks.

No grouping of cases could be shown in any part of the hall. Table 6 shows the theoretical and observed distributions of cases at tables of eight, on each of the two shifts, and for each of the two seating schedules. The observed distributions are remarkably like the theoretical in each case, and suggest no concentration of cases at any group of tables.

Food preparation is by a full-time staff of 36. This group is assisted by 15 student "servers," who help in the kitchen and occasionally help in the actual preparation of food. Service to tables is by 174 student waiters, who work a varying schedule of meals and shifts to allow for class attendance. After preparation, food from large cooking vessels is distributed by the "servers" to smaller dishes, transported by the waiters to the

TABLE 5

Population, cases, and attack rates for infectious hepatitis in grades and classes of total resident and nonresident student body, 1952

Grade or class	Population	Cases	Attack rate (%)
Grade			
7	12	—	—
8	21	2	9.5
9	59	6	10.2
10	68	8	11.8
11	85	6	7.1
12	108	9	8.3
Class			
Freshman	855	62	7.3
Sophomore	532	34	6.4
Junior	363	39	10.7
Senior	330	29	8.8
Graduate	139	6	4.3

TABLE 6

*Observed and theoretical distributions of cases at tables of 8 in the dining hall, on first and second shifts and by two seating arrangements, "X" University, 1952**†

Cases at a table of 8 persons	Tables at dining Shift I			Tables at dining Shift II		
	Seating as of Sept. 3 through Sept. 29†	Seating as of Sept. 30 through Nov. 30†	Theoretical distribution‡	Seating as of Sept. 3 through Sept. 29†	Seating as of Sept. 30 through Nov. 30†	Theoretical distribution‡
0	89	84	89	42	47	42
1	76	81	75	37	31	35
2	25	25	28	14{13	15{14	16{13
3	8{8	8{8	7{6	1	1	3
4	0	0	1	0	0	0
Total	198	198	199	93	93	93
χ^2 =	0.477	1.225		0.364	1.114	
d.f. =	2	2		1	1	
P	+0.75	>0.50		>0.50	>0.25	

* 21 cases, for which no seating data are available, were allocated proportionally, on the basis of the 201 cases for which seating data were available.

† 6 cases, among people who were not assigned seats at the 291 tables under consideration, are omitted from the data for the September 3–29 arrangement. Similarly, 7 cases are omitted from the data for the September 30–November 30 arrangement.

‡ Binomial distribution with

$$p = \frac{\text{number of cases}}{\text{dining hall population}} = \frac{222}{2338} = 0.095$$

TABLE 7

Population, cases, and attack rates for infectious hepatitis in certain groups associated with the dining hall, 1952

Groups associated with dining hall	Population	Cases	Attack rate (%)
Faculty and staff	214	21	9.8
Resident student body	(2112)	(199)	(9.4)
Not employed	1913	157	8.2
Waiters	174	31	17.8
Kitchen servers	15	6	40.0
Hostesses	10	5	50.0
Full-time kitchen employees (32 Negro adults and 4 white adults)	36	—	0.0

tables, and served family style. Ten students act as hostesses, circulating in the hall and overseeing the service, but they do not participate in the food distribution.

The only evidence of epidemic localization which could be detected, other than that already discussed in regard to residents and nonresidents on the campus, occurred in this group of food-handlers. These data are shown in Table 7.

While the attack rate among the resident student body who did not work in the dining hall was 8.2 per cent, it was nearly 18 per cent in the 174 waiters, 40 per cent in the 15 servers, and 50 per cent in the 10 hostesses. For all 199 students working in the dining hall, the attack rate was 21.1 per cent, almost 2.5 times that for the rest of the student body.

No illnesses were recognized in the 36 full-time kitchen employees, all of whom were adults, and 32 of whom were Negroes. All ate from the same foods that were served in the hall, but they and the 15 student "servers" carried their own food to two small dining rooms, one for white and one for Negro employees. Because of age and racial differences, it is particularly difficult to interpret the attack rate in this group.

SEARCH FOR SOURCE OF INFECTION

In the community. The review of city-school absence records did not reveal an unusual amount of illness before or during the college outbreak. Among 3,400 high-school students, a history of illness with jaundice during the preceding 2 months was found in three instances. A review of hospital and infirmary records of a nearby air base showed only three cases of hepatitis in the months of September, October, and November. Sixty-three physicians reported 160 possible cases of infectious hepatitis in the preceding 6 months among the 100,000-odd persons in the city and its outlying areas. About one-half of these cases occurred from August to November. Thus, hepatitis was present in the community, but at no time seems to have exceeded an estimated attack rate of 1 per 1,000 per month.

A small and significant outbreak was detected in the student body of a second small college. On the women's campus of this second college, 5 per cent of the 350 resident students became ill between October 23 and November 15. Viewed in retrospect the clinical findings appeared to be compatible with a diagnosis of infectious hepatitis. None of the 500 students on the men's campus, a mile or so distant from the women's campus, was ill. There is little association between the student bodies of the two colleges; none of the 19 women ill at the second college had ever visited the larger college campus or was closely acquainted with anyone there. A persistent effort was made to establish some common connection between these two college outbreaks, one large and one small, which occurred almost simultaneously, but no such relationship could be determined.

On the campus; earliest case. The earliest recognized onset of hepatitis was on October 4. This case is of particular interest since, in point of time, it could have served as the source of the entire outbreak that followed.

This first patient was a 21-year-old female resident student who complained of anorexia, nausea, vomiting and fever on October 4. She continued her part-time work in the college library, attended classes, went to meals, and slept in her dormitory for several days. On October 12, her symptoms became more marked, and she was admitted to the infirmary. She was discharged on October 25, but was readmitted with a recrudescence of symptoms on November 11.

The patient lived in "Su" dormitory in a room with three other women, none of whom became ill. In the library, she was in casual and transient contact with many students, probably including both nonresident and resident students. She was a member of a literary society, but this society conducted no extramural social activities until

October 25, when the outbreak was well under way. She was unaware of any contact she may have had with a case of hepatitis, either before or after her return to school for the fall semester. Furthermore, no conceivable mechanism could be determined whereby this patient could have disseminated the infection so broadly throughout the college.

On the campus; common environmental sources and vehicles. Since so much evidence pointed to the broad dissemination of the infectious agent uniformly throughout the campus from a single source, much attention was given to water and food supplies.

The water for the entire campus comes from the city mains. It undergoes storage, settling, and two stages of chlorination before entering the city distribution system. It is supplied to the campus through two interconnecting loop mains at a pressure of 50 pounds or more; there is no secondary storage or pumping. At that time, no repair work, alteration, or unusual demand for water could be demonstrated on the campus or along the route of the supply mains. City records showed no significant changes in chemical or bacteriologic analyses. No possible cross connections or other source of contamination could be found; since no building was more than 7 years old, the tracing of pipes and connections was not difficult.

Fresh milk and cream were supplied by a large commercial dairy, and were pasteurized at 160° F for 15 seconds. Twenty-gallon cans used for delivery to the campus were steam sterilized at two stages before use. Examination of dairy records and processes revealed no breaks in techniques of pasteurization or handling. In the kitchen, milk and cream are transferred to open-vat dispensers just before use. There is opportunity for contamination at this point, but it was difficult to visualize a contamination which would lead to such uniform attack rates on each of two shifts during which more than 2,000 persons were served.

A review of menus failed to suggest other food sources of infection except fresh green produce, of which lettuce was the commonest item used without cooking. While no good evidence could be adduced that lettuce was the vehicle of infection, such an hypothesis provides a possible explanation for the simultaneous outbreak at the second college, discussed earlier. Lettuce from the same wholesaler was used by the kitchens of both colleges, and it was acknowledged that occasionally orders from the smaller college may have been filled by "borrowing" from stocks in the facilities where supplies for the large institution were stored. However, a review of invoices failed to substantiate any such transaction during the weeks immediately prior to the outbreaks.

In a retrospective attempt to incriminate food or water as the vehicle of infection, questions regarding consumption of water and certain foods were included in the questionnaire that was circulated on the college campus. The respondents were asked to check columns indicating whether they consume each item "regularly", "occasionally" or "never" when it it served in the dining rooms. Table 8 summarizes the answers to these questions.[4]

Of the five items, "fresh salads" again appears as an interesting item, since there was no hepatitis among the 27 persons who claim "never" to eat fresh salads in the dining room. However, the attack rate among "regular" salad eaters does not differ significantly from the attack rate among those who ate salads only "occasionally" ($\chi^2 = 2.86$, 2 degrees of freedom, $0.30 > P > 0.20$). The distribution of cases by frequencies of consumption of milk, of cream in coffee, and of cocoa do not differ from random.

SEARCH FOR VARIATIONS IN HOST SUSCEPTIBILITY RELATED TO PAST EXPERIENCES

The existence of a large number of people, all of whom had been uniformly exposed to an

[4] As stated above, 2,634 questionnaires were returned. The following table shows the distribution of these questionnaires according to whether or not the respondents ate in the dining hall and according to their illness status.

Ate in dining room	Hepatitis cases	"Questionables"	Persons without clinical hepatitis	Totals
Yes	220	40	2030	2290
No	0	0	333	333
Not stated	0	1	10	11
Totals	220	41	2373	2634

Tables 8 to 13 are based on 2,250 persons (220 with clinical hepatitis and 2,030 without clinical hepatitis) who ate in the college dining room. Those tables do not include the 333 persons who did not eat in the dining room, the 41 questionables, and the 10 others who did not state whether or not they ate in the dining room.

TABLE 8

Relationship between infectious hepatitis and consumption of various items in the dining hall

Item	Frequency of consumption when served in dining hall	Cases	Population	Cases per 100 population
Fresh salads	R*	187	1891	9.9
	O†	31	296	10.5
	N‡	0	27	0
	Total	218	2214	9.8
	(Ns)‖	(2)	(36)	—
Water	R	198	2089	9.5
	O	19	129	14.7
	N	1	10	10.0
	Total	218	2228	9.8
	(Ns)	(2)	(22)	—
Milk	R	130	1219	10.7
	O	32	279	11.5
	N	36	501	7.2
	Total	198	1999	9.9
	(Ns)	(22)	(251)	—
Cream in coffee	R	75	831	9.0
	O	18	132	13.6
	N	100	895	11.2
	Total	193	1858	10.4
	(Ns)	(27)	(392)	—
Cocoa	R	127	1236	10.3
	O	23	237	9.7
	N	45	495	9.1
	Total	195	1968	9.9
	(Ns)	(25)	(282)	—

* R = regularly.
† O = occasionally.
‡ N = never.
‖ Ns = Not stated.

TABLE 9

Relationship between infectious hepatitis and claimed previous history of "yellow jaundice"

Claim history of "yellow jaundice"	Cases	Population	Cases per 100 population
Yes	11	112	9.8
No	203	2080	9.8
Total	214	2192	9.8
(Not stated)	(6)	(58)	—

TABLE 10

Relationship between infectious hepatitis and size of community of residence ("lifetime residents" only) for 930 dining hall patrons of ages 18–22*

Size of community	Cases	Population	Cases per 100 population
Farm	26	258	10.1
1–4,999 persons	10	128	7.8
5,000–24,999 persons	13	152	8.6
25,000–49,999 persons	7	69	10.1
50,000–249,999 persons	23	169	13.6
250,000+ persons	13	154	8.4
Total	92	930	9.9

* A "lifetime resident" in this table is a person who lived in one or more communities of the same size for at least 14 years.

infectious agent, but only 10 per cent of whom had become clinically ill, presented a unique opportunity to evaluate the influence of a number of demographic factors on host susceptibility. The main purpose of the questionnaire was to elucidate certain data in the hope that relationships could be established between probable degree of previous exposure to infectious hepatitis, and susceptibility to the disease (presumably infectious hepatitis) which was epidemic among the college community.

Table 9 summarizes the answers to the question: "Have you ever had yellow jaundice before the recent epidemic?" (In order to eliminate cases of neonatal jaundice the respondent was asked to indicate at what age he had had the disease if his answer was "Yes"; cases of jaundice at early infancy were included in the "No" category.) The answers to this question indicate that previous infection with "yellow jaundice" did not protect those individuals from infection during the epidemic.

Numerous studies have been reported for other diseases in which persons in rural areas are shown to be more susceptible to certain infectious diseases than persons in urban communities. Table 10 shows the results of such an analysis of the residence histories of the "life-time residents" of each size of community among 18- to 22-year-olds who ate in the dining room of the college. This distribution of cases is random throughout six groups of an urban-rural gradient.

It was thought that size of family might influence susceptibility, since members of large families are more liable to exposure to all sorts of infectious agents than members of small families; this is a result of intimate contact with a larger

number of people. Table 11 presents case rates separately for persons with 0, 1, 2, 3, 4, 5, 6+ siblings. Again the distribution of cases is seen to be random. A more detailed analysis was also made by the age of the college person, relative to the ages of his siblings. Again the attack rates did not differ from random.

The questionnaire also asked about exposure to jaundice (other than neonatal jaundice) among members of a person's family. Table 12 summarizes the answers to that question. It is seen that there is a marked excess of cases among those persons claiming previous familial exposure to jaundice ($\chi^2 = 8.64$, 1 degree of freedom, $P = 0.003$).

Table 13 explores the relationship between veteran status and susceptibility. It was thought that veterans, having been subjected to prolonged experiences of mass living and at least occasional episodes of inferior sanitation, should have been less susceptible to the hepatitis virus of this epidemic than the nonveterans. No difference was noted, however.

It is mentioned above that the distribution of cases was essentially uniform over all academic classes and grades. The breakdown by academic class (Table 5) approximates a classification by age. Thus, it can be inferred that the attack rates from ages 13 (eighth grade) to 21 (senior class) were uniform. This observation differs from the usual distribution of infectious hepatitis cases, where the attack rate falls sharply after the ages of 10 to 15 (9).

TABLE 11

Relationship between infectious hepatitis and number of siblings

Siblings	Cases	Population	Cases per 100 population
0	22	223	9.9
1	49	535	9.2
2	53	493	10.8
3	31	325	9.5
4	26	188	13.8
5	13	133	9.8
6+	15	154	9.7
Total	209	2051	10.2
(Not stated)	(11)	(199)	—

TABLE 12

*Relationship between infectious hepatitis and claimed familial exposure to "jaundice"**

Familial exposure to "jaundice" claimed	Cases	Population	Cases per 100 population
Yes	19	105	18.1
All others	201	2145	9.4
Total	220	2250	9.8

* This item was phrased: "If any member of your family has had jaundice, state the approximate year and the person's age." The usual "no" and "not stated" categories are lumped in a "all others" category since negative answers were not requested.

TABLE 13

Relationship between infectious hepatitis and veteran status

Veteran	Cases	Population	Cases per 100 population
Yes	13	151	8.6
No	177	1782	9.9
Total	190	1933	9.8
(Not stated)	(30)	(317)	—

DISCUSSION

While the source and mode of infection could not be determined, there are points of epidemiologic interest in the large outbreak of infectious hepatitis which has been described.

Without doubt, the characteristics of the epidemic curve point to a common source of infection, operating over a short period of time, and uniformly affecting the population resident on the college campus. Widely variant attack rates between campus residents and nonresidents, together with the low incidence of disease in the community at large, help to localize the source of infection to the campus. A careful review of the activities of the patients prior to the outbreak suggests that infection most likely occurred during one of the two time periods, September 25 to October 3, or October 6 to 9.

Among those living on-campus, illnesses were distributed with remarkable uniformity. No localization of the outbreak could be observed in regard to living quarters, dining-hall seating schedules, school grade or college class, or membership in other social groups. The only localization which appeared of possible significance was the disproportionately high attack

rate among student food servers, waiters, and dining-hall hostesses. No convincing explanation for these differences is apparent. Consideration of the nonresident students adds further strength to the evidence for a source in the dining hall. Of the 460 nonresidents, only 12 ate their meals in the dining hall. Of these 12, two had hepatitis; none of the 448 who did not eat in the hall was ill.

While nothing could be found pointing directly to water or milk as in the source of infection, it is perhaps unwise in the case of infectious hepatitis to use the same criteria for the purity of milk and water supplies as would be applicable for less hardy infectious agents. It is possible that milk or water, grossly contaminated with the hepatitis virus, could remain a source of infection after treatment adequate to remove other kinds of agents. The recent epidemic in New Delhi, India (Nat'l. Office Vital Stats., 1956), presumably caused by virus present in water with acceptable coliform counts, is evidence of this.

Possibly the explanation for the outbreak lies in an understanding of its relationship to the epidemic in the smaller school. Although a common use of contaminated fresh produce by the two institutions remains an intriguing possibility, no good evidence substantiates this suggestion. Such an hypothesis sets off a provocative train of thought concerning the problems of sanitary facilities for agricultural field workers, and problems related to the use of treated sewage fertilizers on row crops.

An interesting facet of this outbreak is the apparently complete absence of secondary cases. It is easy and comforting to speculate that further epidemic waves were prevented by the wide use of gamma globulin. While it does not seem likely that the biologic aborted any cases in the primary "wave" of the epidemic, it seems entirely probable that its use may have prevented a second "wave" of cases.

Of great interest are the uniform attack rates among persons of all ages, among persons from both large and small families, among residents of urban areas and of rural areas, among veterans and nonveterans, and among persons claiming and not claiming previous infections with infectious hepatitis. Possible explanations for these are:

A. This epidemic may not have been infectious hepatitis. Lacking virus isolation and/or serologic techniques, it is impossible to be certain that this epidemic was actually caused by hepatitis virus A. However, the findings were completely compatible with those of infectious hepatitis.

B. Infection with hepatitis virus A might not provoke long-term immunity in the host. However, age-specific morbidity rates in general show a decreased incidence with increased age (Sherman and Eichenwald, 1956). This is usually interpreted to mean that circulating antibodies are more prevalent among people of older ages than among young people; this being the result of a greater number of previous clinical and subclinical infections among the older age groups. Furthermore, the success with which pooled human gamma globulin has been used as a prophylactic agent against infectious hepatitis implies that long term immunity does exist (Ashley, 1954, 1955; Stokes and Neefe, 1945; Hsia, Lonsway and Gellis, 1954).

C. The virus causing this epidemic may belong to an immunologically distinct strain of hepatitis virus A (or B) with which the population at risk had had negligible previous experience. The existence of immunologically different strains of hepatitis virus B has been postulated by Havens (1956) as a possible explanation for three hepatitis attacks in a patient, all of which are presumed to be of viral etiology. It is possible that accumulating epidemiologic and laboratory data may lead to a similar immunologic differentiation among the group A viruses.

No satisfactory explanation is offered for the large excess of cases among persons with histories of familial exposures to "jaundice." Perhaps this is related to possible liver damage caused by previous subclinical hepatitis infections which subsequently predisposed those individuals to more severe illness when their livers were insulted by a second infectious agent. This phenomenon has been observed in persons who were infected with hepatitis virus A following an infection with hepatitis virus B (Gauld, 1946). Were this the case, however, the attack rate among persons with histories of clinical "yellow jaundice" should have been at least as markedly increased. This, of course, did not occur.

SUMMARY

An epidemic of infectious hepatitis has been described, involving at least 222 individuals in a college, for a total attack rate of 8 per cent. Cases

were distributed throughout the campus population with great uniformity, with two exceptions: disproportionately low attack rates (0.4%) were observed in students who lived off campus, and disproportionately high attack rates (17 to 50%) were observed in student workers in the common dining hall.

Although every evidence pointed to a common source and mode of infection, these could not be determined with certainty. The outbreak is described as a "case report" of a large and interesting epidemic, the explanation for which remains frustratingly obscure.

Further data are presented which show uniform attack rates among persons of all ages, among persons from large and from small families, among residents of urban areas and of rural areas, among veterans and nonveterans, and among persons claiming and not claiming previous infections with infectious hepatitis. Several possible explanations of these phenomena are discussed.

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FILARIASIS IN DJAKARTA, INDONESIA

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The prevalence of filariasis in Djakarta, capital of Indonesia, has been studied only once. Flu (1929) found nine of 73 nocturnal blood samples positive for microfilaria of *Wuchereria bancrofti*. No one has seemed interested in extending Flu's work, probably because the most disfiguring and disabling feature of the disease, elephantiasis, is rarely seen in Djakarta.

In 1956 the authors started a survey of filariasis in a selected area of the city. This paper is a report of the results of the blood and clinical survey. The results of the entomological study will be published later.

DESCRIPTION OF THE AREA

Djakarta is 6°10′ south of the equator. The temperature is fairly uniform with a mean of about 27°C. The annual rainfall is about 80 inches, heaviest from November through February and usually lowest from June through September. The relative humidity remains uniformly high at about 80 per cent.

The city has two types of living quarters. One consists of stone houses, built along paved streets and located in the center of the city and in the new residential areas. The other consists of smaller houses of wood and bamboo which are built close together along narrow paths and back streets; these are the kampungs of Djakarta. At the edge of the city groups of kampung houses are often separated from each other by small gardens or rice field giving the area a semirural character. In these crowded kampungs, without electricity or running water, people live under unhealthy sanitary conditions and are usually not protected from mosquitoes. Since filarial infections are most likely to be found under such circumstances, we selected for study Rawasari District, a representative area of this type, situated in the Demonstration and Study Centre for Public Health and Preventive Medicine. It is about 6 miles from the sea coast in the eastern region of Djakarta, not far from the place where Flu collected his blood samples in 1929. It is composed of three Kampungs or administrative units: one semirural, the other two typically urban, in none of which malaria occurs. Table 1 presents the population distribution of the three units.

The population of Rawasari District consists of Indonesians, partly indigenous to the area and partly people who have moved in from other sections of Djakarta or from outside the city. They are of the lower socio-economic class, comprising laborers, small venders, *betja* drivers, servants and small farmers. They have no cattle or dogs, but cats are occasionally kept as house pets. Many of the houses have only one or two small rooms.

TECHNIQUE OF BLOOD SURVEY

Blood samples were collected from 8:00 p.m. until midnight by house-to-house visits. As far as possible all persons in each of the houses were examined. With a hemoglobin pipette 20 mm³ of blood were taken from the finger and spread on a glass slide previously cleaned in chromic acid and alcohol. Each slide was used for one blood film only. The smears were allowed to dry on a tray and were then covered to protect them from insects. Next morning the slides were stained with Jaswant Singh and Bhattacharji staining solution (JSBI) and each entire smear examined for microfilariae, recording the total numbers of larvae found.

The name, sex, age and length of residence in the area were obtained from each person examined. They were also questioned concerning elephantiasis and other evidence of filariasis. It was not always possible to obtain the exact age of the people examined, therefore, an estimate of the age was often made by the examiners.

RESULTS OF BLOOD SURVEY

The 7,048 blood samples collected represented more than half of the population of the area. The results are summarized in Tables 2 and 3. Microfilariae of *W. bancrofti* were found in 547 smears (7.8%). No other species were observed. The frequency in males (8.1%) was slightly higher than that in females (7.4%).

Microfilariae in the positive smears were

usually few in number, averaging 28.6 per 20 mm³ of blood. The highest microfilarial count was 251, but the microfilarial count was ten or less in more than 45 per cent of the positive cases.

The youngest child born in the area found positive for microfilariae was 3 years old. We can assume that the minimum time required for microfilariae to appear in the blood is not more than 3 years.

The results of the examination of the 4,311 persons who had lived for more than 3 years in that area are summarized in Tables 4 and 5. In this group the microfilarial rate was 10.4 per cent. The incidence in males was 11.2 per cent and in females 9.7 per cent. The average number of microfilariae per positive smear was 28.3. Among those who were resident for less than 3 years only 3.6 per cent were infected. The slight differences observed in the incidences in the three kampungs were not significant.

TABLE 1

Population distribution and number examined in the area surveyed, Rawasari District

Persons	Kampung			Totals
	Baru-Rawakerbo (Semi-rural)-	Djawa-Rawasari (Urban)	Tjempaka Putih (Urban)	
Total.....	4,789	6,410	2,695	13,894
Examined....	2,313	3,268	1,467	7,048

TABLE 2

Results of blood survey according to age and sex

Age (*years*)	Sex	Persons examined	Positive	
			No.	%
0–1	M	37	0	0
	F	67	0	0
1–5	M	594	9	1.5
	F	543	6	1.1
6–10	M	366	27	7.4
	F	380	34	9.0
11–20	M	709	72	10.2
	F	899	96	10.7
21–30	M	838	53	6.3
	F	937	76	8.1
31–40	M	541	70	12.9
	F	456	29	6.3
41–50	M	224	24	10.7
	F	194	18	9.3
50+	M	146	23	15.8
	F	117	10	8.6
Totals	M	3,455	278	8.1
	F	3,593	269	7.5
Totals	M + F	7,048	547	7.8

TABLE 3

Results of blood survey according to number of microfilariae in 20 mm³ of blood

Microfilarial counts	Persons examined		Microfilariae per 20 mm³
	No.	%	
0	6,501	92.2	0
1–5	168	2.4	441
6–10	83	1.2	633
11–30	156	2.2	2,927
31–50	41	0.6	1,611
50–100	58	0.8	4,119
100+	41	0.6	5,919
Total persons positive...	547	7.8	15,650
Total persons.....	7,048	100.0	
Average infection.....			28.6

TABLE 4

Results of blood survey among people resident for at least 3 years, according to age and sex

Age (*years*)	Sex	Persons examined	Positive	
			No.	%
3–5*	M	268	8	3.0
	F	237	5	2.1
6–10	M	258	22	8.5
	F	277	22	8.0
11–20	M	416	59	14.2
	F	566	77	13.6
21–30	M	455	41	9.0
	F	627	59	9.4
31–40	M	372	61	16.4
	F	328	23	7.0
41–50	M	167	20	12.0
	F	142	25	17.6
50+	M	117	19	16.2
	F	81	8	9.9
Total	M	2,053	230	11.2
	F	2,258	219	9.7
Total	M + F	4,311	449	10.4

* Children born within 3 years are excluded.

CLINICAL SURVEY

Attempts made to obtain clinical data at the time blood samples were taken were not very

successful. Physical examinations were not feasible at night. Although we looked carefully for visible signs of infection such as elephantiasis of legs, arms and other areas, not a single case was observed. Data obtained by questioning the people about fever or lymphangitis were unreliable. However, we gained the impression that the acute phase of the disease was of rare occurrence since the people were not familiar with its symptoms.

TABLE 5

*Results of blood survey among people resident for at least 3 years, according to number of microfilariae in 20 mm³ of blood**

Levels of microfilarial counts	Persons examined		Microfilariae per 20 mm³
	No.	%	
0	3,862	89.6	0
1–5	133	3.1	349
6–10	71	1.7	541
11–30	129	3.0	2,417
31–50	35	0.8	1,371
51–100	50	1.2	3,518
100+	31	0.7	4,486
Total persons positive	449	10.4	12,682
Total persons	4,311	100.0	
Average infection			28.3

*Children born within 3 years are excluded.

A locality in the semi-rural Kampung Baru-Rawakerbo was selected for a more thorough study.

Physical examinations were made on 324 persons, 171 females (109 above and 62 below 15 years of age) and 153 males (92 above and 61 below the age of 15). Blood smears from 271 of these persons showed microfilariae in 32 (11.8%), of whom 15 were females and 17 males.

No cases of acute filariasis or of elephantiasis were seen during these clinical examinations, but painless swellings of the inguinal lymph-nodes were very common. They were found in 54.1 per cent of the females above 15 years of age and in 38.7 per cent of the younger females; in the two male groups they were present in 47 per cent and 47.6 per cent, respectively. In about half of the cases the swelling was present on both sides. The youngest persons with lymph-node enlargement were 2 years old. Most of the enlarged nodes were about 0.5 cm in diameter, but in 22 cases (3 males and 19 females) the swelling was 1.5 cm or larger and in one case it measured 4 cm. Of those who showed microfilariae in the blood, 19 (59.3%) had inguinal lymph-node enlargements, all measuring less than 1 cm in diameter. These were not present in 13 with demonstrable microfilariae.

Of the 239 persons who did not show microfilariae in the blood, 111 (46.4%) had lymph-node enlargement.

Enlarged epitrochlear nodes were found in five

TABLE 6

Results of periodicity survey among hospital patients in Djakarta

Name	Sex	Age (years)	Time of obtaining blood samples and microfilariae found*							
			18	21	24	3	6	9	12	15
Wong	F	19	25.7	97	*176.7*	152.5	87.5	35	3	2.3
End	M	40	3	26.3	*45.3*	42	41	3.7	0	0
Tab	M	24	54	123.3	*137.7*	131.3	118.7	3	0	0
Mud	M	17	1.3	*23*	21.3	19	9.7	0	0	0.7
Gat	M	38	0	1	*1.7*	1.3	1.3	0.3	0	0
Nang	F	60	3.7	7.3	7	*19.3*	10.7	7	0.7	0.7
A. S. Kam	F	40	1	33.5	47.7	*63.5*	41	14	1.3	0.3
Bach	M	30	0.7	4.7	6	*7.3*	1.7	0.3	0	0
Om	M	35	0.7	2	3.7	*8.7*	3.3	2.7	0	0
Suh	F	16	4	27.7	41	*54.3*	39.3	17.3	0.7	1.7
Naz	M	20	2.3	19	35	*37*	36	5.7	1.3	0.7
H. Lim	M	56	16	—	153.3	223.3	*292.7*	127	65.3	18.7
Luk	M	17	0	0	0.3	0.3	*1.3*	1	1.3	0
Sua	F	35	2	3	32.3	28.7	*59.3*	22.7	2	1

* Average in 20 mm³ of blood. Peak numbers are in italics.

males and four females. In four cases these were bilateral. In one case the node was 2 cm in diameter, but the rest were small. Blood smears from eight of these cases were examined but were negative.

Hydrocele, ranging from slight enlargement to the size of an apple, was found in 14 males. The youngest person with this involvement was 20 years old. It was bilateral in two cases. Blood smears from eight of these cases were examined but only one was positive. Five of these cases also had enlarged inguinal nodes and three had a thickening of the spermatic cord, as did two others (14 and 16 years of age) without hydroceles.

PERIODICITY

Microfilarial periodicity was studied in 14 patients found to be positive by routine blood examination at the General Center Hospital in Djakarta. All of these were residents of kampungs and four of them came from localities in the vicinity of the area surveyed. Microfilarial counts were made on 60 mm^3 of blood obtained by finger puncture at 3-hour intervals for a period of 24 hours. Peaks were found at midnight in five cases, at 3 a.m. in six, and at 6 a.m. in three (Table 6).

DISCUSSION

The survey showed that *W. bancrofti* is endemic in the area examined. The microfilarial rate of 10.4 per cent found among those who have lived in the area for at least 3 years is doubtless too low, since single examinations of 20 mm^3 of blood would not reveal all infections, particularly in an area where most of them are light.

The area surveyed is representative of the kampung areas of Djakarta, and hence it is likely that the infection is widely distributed throughout all of them. Evidence to support this view was obtained from the examination of 3,530 blood samples taken at night from patients admitted to the General Center Hospital. Of these, 180 (5.1%) were found to contain microfilaria of *W. bancrofti*. The positives, spotted on a map according to place of residence, proved to be more or less evenly distributed throughout the kampungs in the city. In general the microfilarial counts were low, which probably explains the absence of acute symptoms and of elephantiasis in the persons examined during the survey. On the other hand, hydrocele was not infrequently discovered in the clinical examinations. Hospital records do not give a true picture of this involvement since people in Djakarta consider hydrocele a result of continuous hard work rather than a manifestation of disease, and usually do not seek medical aid unless the swelling is so large as to cause considerable trouble. None of the 14 cases found in the survey accepted proffered medical aid. Records of the General Center Hospital over a period of 5 years (1952 to 1956) showed that 220 hydroceles had been operated on. Most of these cases came from the various kampung areas of the city; an indication that bancroftian filariasis is endemic in all.

Lymph-node enlargement was a frequent finding in the people examined in the survey area. However, we must be cautious in attributing this to filariasis. Such enlargement is not characteristic of areas where the periodic form of *W. bancrofti* is prevalent, and it is well known that inguinal nodes are often seen in people who go barefoot. Probably most of the enlarged inguinal nodes found in this survey were not related to filarial infection. The etiology of the epitrochlear lymph nodes is also uncertain. For this reason both these conditions should be disregarded in arriving at the filariasis rates in the population surveyed. If only microfilaremia, hydrocele and thickening of the spermatic cord are taken into consideration, the following rates are obtained for the area in which the clinical survey was carried out: filarial infection, 11.8 per cent; filarial disease, 3.3 per cent; endemicity, 14.4 per cent.

SUMMARY

Wuchereria bancrofti of the nocturnal type is endemic in the kampung areas of Djakarta. In a representative area the filarial infection rate of 4,311 persons resident there for at least 3 years was 10.4 per cent. Men showed somewhat more infection than women, but the difference was not significant. The number of microfilariae found in the blood was usually small, the average infection being 28.3 microfilariae in 20 mm^3 of blood, but the microfilarial count was 10 or less in more than 45 per cent of the cases.

In the area examined no elephantiasis was found. Generally it is rarely seen in Djakarta. On the other hand, hydrocele was found in 14 of the 92 men (15.2%) examined above 15 years of age. The youngest person showing hydrocele was 20 years of age. Thickening of the spermatic cord

was found five times. The youngest person showing this abnormality was 14 years of age.

Lymph-node enlargement was frequently found in both men and women, but there is no certainty as to the etiology of these enlargements.

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THE HISTOPATHOLOGY OF EXPERIMENTAL INFECTIONS IN MICE WITH *SCHISTOSOMATIUM DOUTHITTI*[1]

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In previous reports on the experimental histopathology of *Schistosomatium douthitti*, the penetration of cercariae into the skin of normal and previously infected mice (Kagan and Meranze, 1955) and the changes in the liver following infection and chemotherapeutic cure (Kagan and Meranze, 1957) were described. In this final phase of the project the spleen, intestine, lungs, bone marrow, and lymph nodes were studied.

The general literature on the histopathology of clinical and experimental schistosomiasis is too vast to be reviewed in this paper. References reviewing zoological and clinical schistosomiasis to the year 1931 may be found in Khalil (1931), and for the years 1931 to 1948 in Bouillon (1950). In recent years workers in Brazil and Venezuela have published excellent papers on the histopathology of clinical and experimental schistosomiasis. Gelfand's monograph (1950) on a clinico-pathological study of South African schistosomiasis reviews many of the papers dealing with the clinical pathology of this disease in Africa.

MATERIAL AND METHODS

The sectioned material used in this study was essentially similar to the material described in our earlier paper (Kagan and Meranze, 1957). Briefly, 78 mice were divided into five groups. The first group (17 mice) received 50 cercariae, which developed into male and female worms, and was necropsied from 10 to 393 days after exposure. The second group of eight mice was infected with female worms and was necropsied 15 to 150 days after exposure. In a third group, 17 mice were exposed to 50 to 300 male cercariae and was necropsied 15 to 840 days after exposure. In group four ten mice, initially infected with 50 cercariae, were challenged with approximately 100 cercariae 60 days after infection and were necropsied, one each day, on 10 successive days. Ten control mice were infected for the first time with 100 cercariae of *S. douthitti* and were necropsied simultaneously with the challenged group. Seven mice (group five) were cured by chemotherapy after exposure to *S. douthitti* (Kagan and Meranze, 1957) and studied at various intervals after cure. In addition, studies were made of a few mice exposed to infections by *S. japonicum* and *S. mansoni*, and of sections of the lung of a young rat infected with *S. douthitti*.

Tissues were removed from animals killed by a blow on the head and placed quickly in Helley's Maximow-Zenker-formol (9 parts Zenker's solution without acetic acid and 1 part neutral formalin) for 12 hours. After celloidin embedding, sections were cut at 10 microns and stained with dilute Delafield's hematoxylin and counterstained with eosin and azure II.

DISCUSSION OF EXPERIMENTAL RESULTS

Spleen. The normal mouse spleen is composed of white and red pulp. The white pulp lymphatic tissue is concentrated in periarterial sheaths. Within the white pulp are lymphatic nodules which occur usually at the periphery of the periarterial sheaths. In these nodules are germinal centers composed of actively dividing lymphocytes. Surrounding each nodule is a marginal zone of medium-sized and small lymphocytes. The red pulp occupies about one-third the volume of the spleen. Within the red pulp occur numerous, small, active foci of erythropoietic tissue. These areas are concentrated subjacent to the capsule of the organ.

[1] This research was initiated during the tenure of a National Research Council Postdoctoral Fellowship in the Department of Microbiology, University of Chicago. I wish to thank Miss Alice Roberts for preparation of the slides. This project was also aided in part, by a grant (E586) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service.

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For a more complete description of the spleen, reference should be made to Singer (1954).

Damage to the spleen in experimental schistosomiasis varies with the experimental host and the schistosome species. Brumpt and Chevallier (1931) described lesions in the spleens of mice infected with *S. haematobium* and *S. bovis*. Eggs were very abundant, but no adult worms were found. Meleney *et al.*, (1953) observed lesions in the spleen of mice infected with *S. mansoni* similar to those seen in the liver. Fairley, Mackie and Jasudason (1930) reported that the spleen was rarely involved in goats infected with *S. spindalis*. Coelho (1954) reported that lesions could not be discerned in the spleen of rats naturally infected with *S. mansoni* in Brazil. Coelho and Magalhães (1953) observed no eggs in the spleens of monkeys, *Cebus sp.*, naturally infected with *S. mansoni*. Koppisch (1937) reported no lesions or eggs in the spleen of rats and rabbits experimentally infected with *S. mansoni*.

In the present study splenic sections from mice infected with male and female worms revealed eggs 30 days after infection. Lesions were not localized in any one area of the spleen. In the series infected with female worms, no eggs were seen after 150 days of infection (end of experimental series) and no gross pathology was evident. In the series infected with male worms a typical pseudotubercle was formed in the spleen around a dead worm. In addition, the spleen of chronically infected mice showed congestion and an apparent increase of red pulp.

Three general types of inflammatory reactions were noted around eggs in splenic tissue with our material. In a mouse infected with *S. mansoni* two typical pseudotubercles, similar to the type seen surrounding eggs in the liver, were found around isolated eggs (Fig. A). In mice infected with *S. douthitti* there was no marked circumscription of connective tissue around eggs (Fig. B). In *Peromyscus maniculatus*, infected for 626 days with *S. douthitti*, eggs surrounded with inflammatory cells were found in the spleen but there were no pseudotubercles (Fig. C). Mice challenged after 60 days of infection showed no enhanced cellular response. In the spleens of mice cured of their infections, the return to normal was not as marked as in the liver (Kagan and Meranze, 1957); eggs were still apparent 97 days after cure but were not normal in appearance (Fig. D).

Accumulations of eggs in the spleens of mice infected with *S. japonicum* were also common and the gross pathology was similar to that of *S. douthitti* infections. In marked contrast, the eggs of *S. mansoni* rarely collected in great numbers in the spleen, and whitish spots under the capsule have never been observed. Adult worms of *S. douthitti* have been recovered in the splenic veins and inside the spleen of mice in our laboratory. Only one section showed the presence of an adult male worm in the spleen.

No marked change in the density of lymphatic nodules or any evidence of lymphocytic hyperplasia was evident in infected mice.

The normal spleen of the mouse undergoes great variation in size since it is an erythropoietic organ (Dunn, 1954). At necropsy five of our 78 mice had greatly enlarged spleens, one mouse a small atrophied spleen, and one mouse a small, very hard spleen. At necropsy, in general, the spleens of mice infected with *S. douthitti* are slightly enlarged and many whitish spots are macroscopically evident under the capsule.

Small intestine. Histologically, the intestine is divided transversely into the following areas: serosa, outer longitudinal and inner circular layers of muscle, submucosa, muscularis mucosae and mucosa (Fig. E).

Surour (1928) pointed out the importance of the muscularis muscosae in controlling the pathology of the disease in the intestine and in egg deposition. He recognized a "supramuscularis mucosa ova deposition" and an "inframuscularis mucosa ova deposition." The formation and growth of bilharzial papillomata in the mucosa and the attendant breakdown of the epithelial elements was believed to be responsible for freeing eggs from the intestine. Inframuscularis mucosa egg deposition may lead to deep ulcer formation. Faust and Meleney (1924) reported that, in dogs infected with *S. japonicum*, schistosomal abscesses in the wall of the intestine always contained eggs. Koppisch (1937) reported marked congestion of mucosal and submucosal veins of the ascending colon of rabbits 40 days after infection. Eggs in the mucosa and submucosa and ulceration of the mucosa were never observed. Fairley, Mackie and Jasudasan (1930) observed a bilharzial colitis in goats infected with *S. spindalis*. They

reported a patchy enteritis and scattered petechial hemorrhages on the mucosal surface of the small intestine. Coelho and Magalhães (1953), in experimental infections of *Cebus* monkeys, found worms *in copula* in the serosa of the intestine. Many eggs were found in the mucosa and submucosa. Coelho (1954) found more eggs in the small intestine of naturally infected rats than in the large intestine. Intense inflammatory reactions were noted in the deeper portions of the mucosa. Coelho and Coutinho (1955) reported granulomatous inflammatory reactions in the mucosa of a naturally infected opossum.

In our study, eggs were found 34 days after infection, in the mucosa and submucosa of mice infected with male and female worms. After 60 days a general inflammatory response with abscesses in the mucosa, submucosa and mesenteric fat was evident. The muscle coat was compressed and in places appeared to have broken through (Fig. F). Masses of eggs were seen in the lamina propria of the villi. At 106 days intense inflammation was evident with fibrous tissue circumscription and pseudotubercle formation. At 120 days the main inflammatory response was found in the muscle layer, submucosal layer and mesenteric fat. The mucosa was infiltrated with inflammatory cells. Marked thickening of the muscle coat was evident (Fig. G). The area primarily involved was the inner muscle layer. This may be due to the fact that eggs probably entered the intestine through small capillaries which empty into the inner muscle layer. It appeared as though the eggs first invade this area and then traverse a plane between the outer and inner muscle layer. The muscularis mucosae appeared to act as a temporary mechanical barrier to the inflammatory process because one saw, repeatedly, involvement of the submucosa and not the mucosa. Adult worms may actually breach the serosa of the intestine mechanically (Fig. H) and the mesenteric fat membrane may be extensively involved with eggs and inflammatory nodules.

Eggs may infiltrate mesenteric lymph nodes. In mice infected with *S. japonicum* there was a tremendous thickening of the muscle wall and the submucosa with little or no involvement of the mucosa. Large numbers of eggs invaded the mucosa and lamina propria of the intestinal villi (Fig. I).

In sections from mice infected with female worms similar inflammatory reactions were observed around clusters of eggs. In the mesenteric fat there was no evidence of inflammation around adult worms. After 60 days of infection eggs were found in the mucosa and submucosa. Surrounding the eggs were accumulations of eosinophils, lymphocytes and heterophils. The reactions around these eggs were focal. By 90 days a general inflammatory response was apparent in the mucosa and mesenteric fat. Areas of the muscle coat were broken through and there was much focal involvement around the eggs. At 120 days the inflammatory response in the mesentery increased. At 150 days there appeared to be less inflammation. The muscle coat around the intestine had hypertrophied and was perhaps containing the infection.

In a mouse heavily infected for 23 days with male and female worms, vascular engorgement of the villi was evident, accompanied by marked inflammation and extensive superficial necrosis of the mucosa. A longitudinal section of the mucosa showed many areas with free blood cells. After drug treatment a great deal of focal involvement in the mucosa and serosa took place around accumulations of eggs. In some areas there appeared to be repair of the muscle coat. Areas of involvement were well circumscribed.

In none of our slides was ulceration of the intestine apparent. In laboratory infections, heavily infected mice generally die of intestinal hemorrhages. The cecum and large intestine are usually full of clotted blood in the dead animal. Living worms accumulate in large numbers in the large vein that borders the edge of the cecum.

Lungs. The respiratory portion of the lung, in sections, appears as a lacework of spaces separated from one another by septa. Bronchioles, blood vessels and septal cells are distributed within the septa.

Fairley (1920) found that monkeys infected with *S. haematobium* had extensive pulmonary involvements caused by both adults and ova, which terminated in an interstitial pneumonia. *S. haematobium* was more reactive in this regard than *S. mansoni*. Gelfand (1950) reviewed the literature of pulmonary bilharziasis and confirmed Fairley's early observation that, in human schistosomiasis, ova of *S. haematobium* are found more frequently in the lungs (60%) as compared to *S. mansoni* (12%). Surour (1928)

reported the presence of localized tubercle-like lesions and diffuse cellular lesions in the lungs of persons. After the injection of living eggs in the ear vein of rabbits typical "experimental bilharzial tubercles" were found. The injection of dead eggs led to no tubercle formation. Koppisch (1937) made a careful study of the lesions in the lungs of experimentally infected rabbits and rats. He believed that the schistosome maintained its intravascular position, entering the air spaces of the lung only on rare occasions. Inflammatory responses against adults, young schistosomulae, and eggs were reported. Faust and Meleney (1924) reported no eggs in the lungs of experimentally infected dogs. Meleney *et al.*, (1953) reported that after 8 weeks, *S. mansoni*, *S. japonicum*, and *S. haematobium* eggs and adult worms were found in lungs of experimentally infected mice. In rabbits, after 8 to 10 weeks of infection with *S. japonicum*, acute egg abscesses with localized pneumonitis, central necrosis and giant cell formation were evident. Coelho and Coutinho (1955) reported pseudotubercle lesions in lungs of experimentally infected monkeys. Coelho (1954) reported that in naturally infected rats intensive lesions caused by dead worms caught in the vascular net in the lung were frequently found in all animals examined. Many of these lesions were similar to lesions described from human infections. Coutinho (1954) reported that all lesions in experimentally infected (*S. mansoni*) guinea pigs were primarily in the arterial system of the lungs and were characterized by necrosis which had a direct relationship to the dead worms in the arterial branches and was not due to toxins liberated by living worms. Kagan (1953) reported that the lungs of young monkeys experimentally infected with large numbers of cercariae of *S. douthitti* showed hemorrhage and extensive damage.

In mice exposed to 50 cercariae the inflammatory response around eggs of *S. douthitti* was first evident after 180 days. In general, the lungs of lightly infected animals showed very few eggs or signs of inflammation due to the presence of the worms. In sections from mice infected with male worms numerous foci or mononuclear inflammatory cells were observed surrounding small blood vessels.

In the series infected for 60 days before challenge, a larger number of cercariae was used. In these sections numerous lesions consisting of eggs in the center of a small circumscribed inflammatory nodule was apparent (Fig. J). Mature, female worms were found in the lungs with no apparent cellular response to their presence. Large areas of necrosis and infarction were noted around dead worms. This was especially true in the lungs of a rat infected with *S. douthitti* (Fig. K).

Young schistosomulae migrating through the lung were observed in two instances 3 and 4 days after exposure. In one mouse the cercariae appeared to be lying free in the alveolar space very close to the edge of the lobule. In the second instance the schistosomula appeared to be lying in the tissues near a bronchiole (Fig. L). No cellular response was evident in either instance.

Bone marrow, kidney and lymph nodes. The histology of the bone marrow, kidney and lymph nodes in infected animals was very similar to that of these tissues in normal uninfected controls. In early stages of the bisexual infection there was only a suggestion of a hypergranulocytosis in the bone marrow and there was no marked depletion or replacement of cells of the erythropoietic series. In mice infected with female worms and those infected with male worms the bone marrow did not appear to differ from the normal.

The kidney showed no schistosomal lesions. A moderate, occasionally marked, lymphocytic infiltration was seen about blood vessels and glomeruli. The above characteristics were most prominent in the combined male and female infected series and not as prominent in the male or female series.

Some lymph nodes showed congestion and sinus hyperplasia. Some Peyers' patches and mesenteric lymph nodes were infiltrated with eggs. In lymph nodes close to concentrations of eggs, a slight infiltration with granulocytes was apparent. In general, however, the lymph nodes were normal in appearance.

GENERAL DISCUSSION

Our observations of the host response to the eggs of *S. douthitti* and *S. mansoni* in the spleen of *Mus musculus*, and the response in the deer mouse, *Peromyscus maniculatus*, against eggs of *S. douthitti*, may have a bearing on the general problem of host-parasite specificity and parasite survival. The inflammatory response in the deer mouse (*P. maniculatus*), in some parts of the country a natural host for *S. douthitti*, was not as intense or of the same character as the response

in the white mouse. The inflammatory response evoked by eggs of *S. mansoni* in the spleen was similar to the reaction seen in the liver. This has been reported previously by Meleney *et al.*, (1953). The white mouse is more resistant to infection with *S. mansoni* than to infections with *S. douthitti* (Olivier, 1952). In his study the rate of infection with *S. mansoni* varied from 10 to 30 per cent, against approximately 50 per cent for *S. douthitti*. The response of the spleen to the egg of this parasite reflects the host-parasite balance.

We observed no mechanism for the release of eggs from the intestine of the mouse. Hemorrhage was noted in heavily infected animals but ulceration of the mucosa was not apparent. The fact that eggs are carried into the lamina propria of the villus may be an indication of the escape pathway in this host. Goodchild (1956) reported passage of ligatures through the wall of the intestine into the lumen of frogs by a process of dedifferentiation of the cells near the ligature. Perhaps the same mechanism is operative in schistosomiasis with passage of eggs into the lumen of the intestine by a mechanism similar to diapedesis or by histo-degeneration coupled with rapid recovery to near normal histology after the passage of eggs.

The apparent extra-alveolar position of migrating schistosomulae lends credence to the hypothesis that migration of the worms from lungs to liver may involve an extravascular route (El-Gindy, 1950). We have recovered many young schistosomulae in the chest cavity of heavily infected mice 2 to 4 days after exposure (unpublished results).

The lack of a distinct cellular response in the bone marrow was unexpected. There was also no sign of increased hematopoiesis in the spleen. This may be due to the fact that mice were very lightly exposed. The absence of a marked cellular response in the tissues of the immune-challenged series was also disappointing. The fact that animals were necropsied within 10 days after exposure may explain why we were unable to observe changes in the tissues of these animals.

The lack of lymphocytic hyperplasia or increase in number of nodules in the spleen may indicate that the cellular response to this metazoan infection differs from that elicited by protozoan species (Taliaferro, 1956).

SUMMARY AND CONCLUSIONS

The experimental histopathology of the spleen, intestine, lungs, bone marrow and lymph nodes were studied in mice infected with *Schistosomatium douthitti*. The inflammatory response in the spleen toward eggs was not as circumscribed as noted in typical pseudotubercles surrounding eggs in the liver. No evidence for an enhanced hematopoietic response or increase in lymphocytic nodules in the white pulp was discerned.

In the intestine eggs were found in the submucosa and mucosal lamina propria. Thickening of the serosa and the inhibiting action of the muscularis mucosa in confining eggs and the inflammatory response to the submucosa and muscle layers were noted. In no instance were abscesses of the mucosa seen. In the lungs inflammatory nodules surrounding eggs were observed 60 days after infection. Mature, living worms were seen in the lungs eliciting no apparent cellular response. The histology of the bone marrow, kidney and lymph nodes in infected animals was very similar to that of tissues in the normal, uninfected controls.

Comparison of lesions caused by eggs in the spleen of *Peromyscus maniculatus*, (deer mice) and lesions caused by eggs of *S. mansoni* in white mice indicated differences in the cellular response of those hosts and may be a reflection of the mechanism of parasite survival.

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PLATE I

FIG. A. Mouse spleen with pseudotubercles around single egg of *S. mansoni*

FIG. B. Mouse spleen with eggs of *S. douthitti*

FIG. C. Spleen of *Peromyscus maniculatus* with eggs of *S. douthitti*

FIG. D. Mouse spleen 97 days after chemotherapeutic cure

FIG. E. Normal histology of mouse intestine. Note the muscularis mucosae

FIG. F. Breakdown of the muscle layer in the intestine of a mouse infected with *S. douthitti*

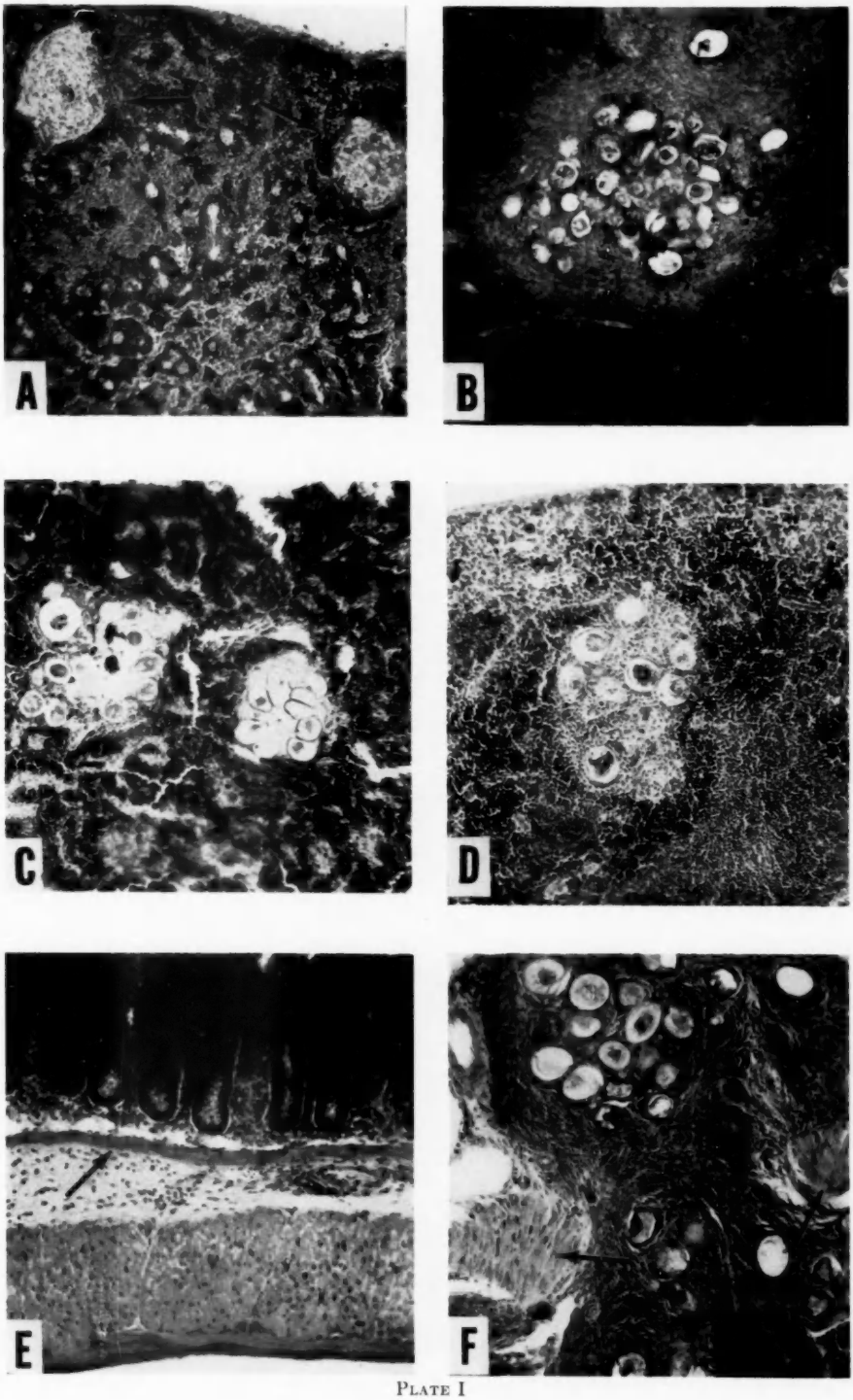


PLATE I

PLATE II

FIG. G. Hyperplasia of the intestinal serosa of a mouse infected with *S. douthitti*

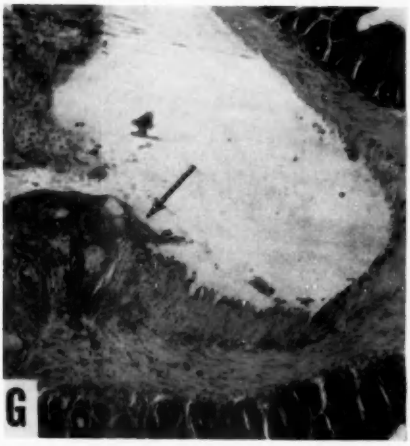
FIG. H. Penetration of the submucosa of the intestine by an adult worm

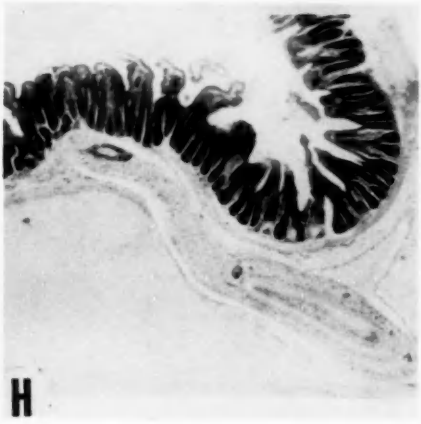
FIG. I. Infection of mouse with *S. japonicum*. Note huge accumulations of eggs in lamina propria of the villi.

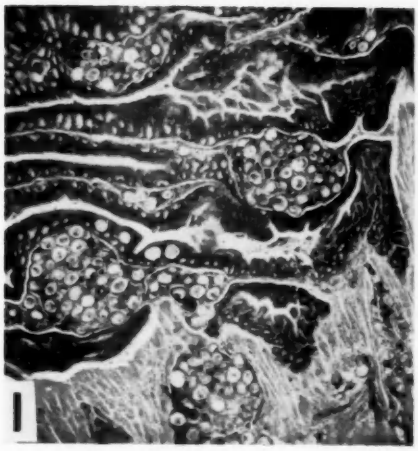
FIG. J. Inflammatory nodule surrounding egg of *S. douthitti* in lung of mouse

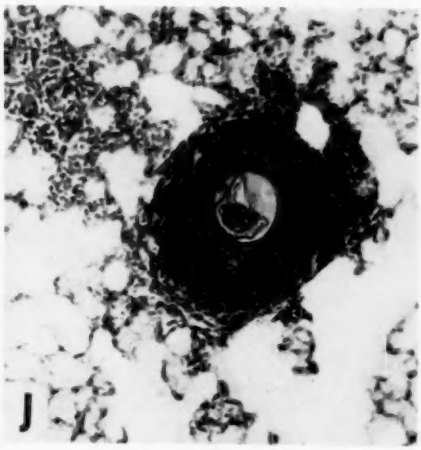
FIG. K. Pseudotubercle around adult worms in lung of rat infected with *S. douthitti*

FIG. L. Cercariae of *S. douthitti* in lung of mouse 4 days following infection









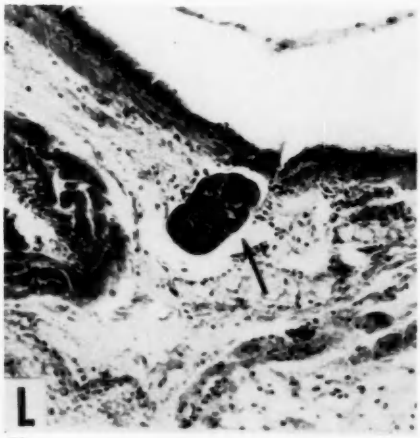


PLATE II

LABORATORY SCREENING OF COMPOUNDS FOR MOLLUSCICIDAL ACTIVITY AGAINST *ONCOMELANIA NOSOPHORA* WITH AN IMMERSION TEST AND A MODIFIED PLATE TEST

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In regions where schistosomiasis is endemic, public health officials have recognized that destruction of the snail intermediate hosts is an important aspect of control of the disease. Consequently, a number of agencies have set up procedures for the laboratory-screening and field-testing of potential molluscicides. Effective compounds have been found but their full scale use often is not economically feasible. For this reason the search for better molluscicides should be continued.

Until recently, laboratory screening of chemicals for molluscicidal activity against *Oncomelania nosphora* consisted of the plate test (McMullen, 1949, 1951). This test was designed to simulate the moist soil habitat to which molluscicides are usually applied. It has been found that some compounds that appeared to be effective in the plate tests were of little value under field conditions (McMullen, 1952 and Prof. Rept., 406th MGL, 1955). Since field tests are expensive and time-consuming, a more critical laboratory screening test would be useful. When aquatic snails were used in screening potential molluscicides (Kuntz and Stirewalt, 1950; Kuntz and Wells, 1951; Kuntz, 1952; Nolan, Bond and Mann, 1953; Bond and Nolan, 1954 and Vallejo-Freire, *et al.*, 1954), immersion tests gave good results. In the present report a standardized immersion test and a modified plate test have been compared.

PROCEDURES

Immersion test. Stock solutions were prepared by dissolving 0.1 gm of active ingredient in 100 ml of solvent (acetone or distilled water). The stock solutions were dispensed into 20-ml vials. A series of four twofold dilutions containing 0.025 mg (1.25 ppm), 0.05 mg (2.5 ppm), 0.1 mg (5 ppm) and 0.2 mg (10 ppm) of active ingredient was obtained when the vials were filled with water. Solvents other than water were evaporated and replaced with distilled water. Each dilution was set up in duplicate and ten snails of average adult size were placed in each vial. Only snails which had emerged from their shells in a dish of distilled water were used. The vials were filled to the brim with distilled water and covered with glass slides to prevent the snails from leaving the solution. After six hours in the chemical, the snails were rinsed with six changes of distilled water and placed in Petri dishes for 66 hours. At this time the shells were crushed and the numbers of living snails recorded. Control series for each experiment were not set up because it had been observed on numerous occasions that immersion in distilled water had no deleterious effect on the snails.

Plate test. The plate test was modified to insure use of known amounts of chemical in four fivefold dilutions and was run simultaneously with the immersion test. The same stock dilutions were used in both tests. Varying amounts of chemical in 2-ml aliquots were spread evenly on filter paper in large Petri dishes and set out in duplicate. Ten adult snails were placed on the center of the filter paper after 3 ml of water had been added to moisten the impregnated paper. Twice a day the snails were centered on the filter paper and water lost by evaporation was replaced. After 96 hours the numbers of living snails were recorded.

All tests were carried out at 28 to 30°C. The 50 per cent endpoint (LD_{50}) for all tests was calculated by the Reed and Muench (1938) method. Sodium pentachlorophenate (Monsanto Chemical Co.'s Santobrite) and dinitro-o-cyclohexylphenol (Dow Chemical Co's DN-1 Dry Mix), considered to be the most efficient molluscicides in use, served as controls.

We are indebted to Dr. D. B. McMullen for forwarding the compounds from the Monsanto Co., R. T. Vanderbilt Co., and B. F. Goodrich Company. The Dow Chemical Co., E. I. Dupont de Nemours Co., Rohm and Haas Co., and Onyx Oil and Chemical Co., supplied chemicals at our request. Some of these compounds had been found toxic for an intermediate host of *S.*

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TABLE 1

Compounds with an LD_{50} against O. nosophora *of less than 0.1 mg by immersion test or 0.2 mg by plate test*

Chemical	Supplier	Immersion test		Plate test	
		mg	ppm	mg	ppm
CP 362	Monsanto	.100	5.	.183	92.
CP 369	Monsanto	.063	3.	.223	110.
CP 2289	Monsanto	.155	7.7	.084	42.
CP 2291	Monsanto	.118	5.9	.036	18.
CP 4743	Monsanto	>.200	—	.135	67.
CP 5470	Monsanto	>.200	—	.085	42.
CP 5478	Monsanto	.174	8.7	.155	77.
CP 5479	Monsanto	.170	8.5	.035	17.
CP 5710	Monsanto	.191	9.5	.165	82.
CP 6637	Monsanto	>.200	—	.196	98.
CP 6641	Monsanto	.100	5.	1.040	520.
CP 7111	Monsanto	>.200	—	.070	35.
CP 7112	Monsanto	.137	6.8	.044	22.
CP 4728	Monsanto	>.200	—	.176	88.
CP 4737	Monsanto	.025	1.25	.016	8.
CP 6057	Monsanto	.109	5.5	.158	79.
Vancide F 1472	R. T. Vanderbilt	>.200	—	.200	100.
Premerge	Dow	.036	1.8	—	—
Weedkiller	Dupont	.039	1.95	—	—
2,4,5-Trichlorophenol	Dow	.025	1.25	—	—
Dinitro-o-sec-amylphenol	Mellon Inst.	.020	1.	.080	40.
Dinitro-o-sec-butylphenol	Dow	.013	.65	.080	40.
Dinitro-o-cresol		.054	2.7	—	—
2-phenyl-4,6-dinitrolphenol		.048	2.4	—	—
Control chemicals					
Sodium pentachlorophenate	Monsanto	.028	1.4	.066	33.
Dinitro-o-cyclohexylphenol	Dow	.028	1.4	.017	8.5

TABLE 2

Compounds with low toxicity against O. nosophora

Chemical	Supplier
49 Compounds	Monsanto
Onyxide	Onyx Oil and Chemical
Ammonox G	Onyx Oil and Chemical
Ammonox TRM	Onyx Oil and Chemical
Ammonox DME	Onyx Oil and Chemical
BTC 471	Onyx Oil and Chemical
Hyamine 10X	Rohm and Haas
Hyamine B104	Rohm and Haas
Hyamine 1622	Rohm and Haas
Hyamine 2389	Rohm and Haas
Vancide F 1501	R. T. Vanderbilt
Vancide F 1512	R. T. Vanderbilt
Vancide F 1565	R. T. Vanderbilt
Strobane	B. F. Goodrich
Malathione	Ihara Noyaku

mansoni by other laboratories (Vallejo-Freire et al. 1954). The new compounds were tested as part of a routine screening program.

RESULTS AND DISCUSSION

The LD_{50} for each of 89 chemicals was determined by the immersion test and for 75 of these compounds by the modified plate test. Twenty-four of these compounds which had an LD_{50} of 0.1 mg (5 ppm) or less by the immersion test and/or 0.2 mg (100 ppm) or less by the plate test are listed in Table 1. The results obtained from more than 20 tests with the two control compounds have been included in this table. The compounds in Table 2 had a higher LD_{50} and are not considered toxic enough to warrant further testing. Four chemicals had a lower LD_{50} in the immersion test than the control compounds. However, three of these: dinitro-o-sec-amylphenol; 2,4,5-trichlorophenol and dinitro-o-sec-butlyphenol, have given poor results

in field tests. CP 4737 should be tested in the field.

Although the present immersion and plate tests are well standardized, they are not completely satisfactory in that they do not always give reproducible results. This is probably associated with variations between different lots of snails and the handling of the snails rather than the technical deficiency of the tests themselves. Snails must be handled in the same manner each time a test is made. For example, unless the snails were first placed in water and only those that emerged were used, the LD_{50} of the control compounds was high. On eight occasions both control compounds weren used i the same test and in seven of these there was close agreement in the results.

The plate test has several variables which are eliminated in the immersion test. In the plate test snails may be in contact with the chemical for a long period (96 hours) and during this time some snails ingest considerable amounts of impregnated filter paper. The precise effect of ingested chemicals on the snails over a 4-day period is not known, but this does not increase the accuracy of a laboratory experiment. With the less irritating compounds the snails sometimes move off the moist filter paper and after drying they retract into their shells. Thus the snails are not in contact with the chemical until they are centered on the moist paper and again emerge from their shells. With the more irritating compounds the snails sometimes retract soon after their initial exposure and do not emerge again. In the immersion test each snail is submerged in toto for only six hours and these discrepancies are minimal. This method also eliminates the misleading impression obtained when plate-test results are reported in ppm or in terms of a dilution. Since the amount of water in the plate varies continuously, the concentration of the chemical also varies. Therefore, an LD_{50} in ppm obtained with the plate test is not analogous with that reported by other laboratories employing immersion-type tests against aquatic snails.

On occasions when there was a significant difference between the results of simultaneous plate and immersion tests, both were repeated and it was evident that the immersion test was more readily duplicated than the plate test. It is believed that the immersion test should replace the plate test for screening molluscicides against *O. nosophora*.

SUMMARY

An immersion test for screening chemicals for molluscicidal activity against *Oncomelania nosophora* snails and a modification of the McMullen plate test are described. Results of screening 89 compounds by these methods are given. Twenty-four compounds had an LD_{50} of less than 0.1 mg (5 ppm) by the immersion test and 0.2 mg (100 ppm) by the plate test. Sodium pentachlorophenate and dinitro-o-cyclohexylphenol were control chemicals. It is believed that the immersion test eliminates several variables inherent in the plate test and that it is preferable for screening of molluscicides in the laboratory.

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INTESTINAL PROTOZOA AND HELMINTHS IN THE PEOPLES OF WESTERN (ANATOLIA) TURKEY[1]

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By invitation of the Minister of Health of the Turkish Government, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, sent a field group to western Turkey for a period of 2 months in the summer of 1953. This group, consisting of 2 investigators (medical zoologist and parasitologist) and 3 assistants, was designated as the U. S. Naval Medical Reconnaissance Group to Turkey. This trip was made as a continuation of geomedical and biological studies (Kuntz, et al., 1955; Lawless, et al., 1956; and Wells, 1956) in the Near East with emphasis on the parasites of man and animals, and their host-parasite and/or-vector relationships.

During the course of this tour from the Bosporus to Ankara a total of 349 human stools were taken at four different localities. A study of this material has provided data for the present report.

MATERIALS AND METHODS

Several unsuccessful attempts were made to obtain stool specimens from villagers in the areas visited. Therefore, it was necessary to solicit the assistance of military officials in camps at different localities. The majority of stools were obtained from recruits of the Turkish Army, 18 to 30 years of age, camped in four representative areas:

Istanbul, a large, port city.

Sapanca, a village with a population of 1,500 to 2,000 persons, 45 miles inland from Istanbul, located on the shore of a small freshwater lake. This area is a part of the fertile coastal plain where the majority of the people are engaged in farming.

Bolu, a city of 8,000 people part way up the western slope of a mountainous area in which most of the people are engaged in farming and sheep herding. Some of the families are associated with lumber camps.

Ankara, the capital city with a population of 300,000 located in the western part of the elevated central plateau. Most of the peoples outside the city are farmers; many associated with sheep herding and the wool industry.

Men from whom stools were obtained did not necessarily represent the peoples of the immediate vicinity since some had come from distances greater than 100 miles. A few within the preceding year had returned with Turkish military units which had taken part in the Korean campaign. Fecal specimens were taken on a 'demand' basis at the time of morning muster. With the aid of an interpreter a brief history, including recent employment, locality of home, and travel during the previous 3 years, was obtained from each person. As in our previous surveys, fecal samples were fixed by the "MIF" method (Sapero and Lawless, 1953) since materials preserved in this manner are well stained and fixed immediately and can be examined at a later date with every expectation of obtaining reliable identifications of protozoa and helminths. The majority of specimens were fixed within 1.5 to 2 hours after passage.

Fifteen-ml capacity, screw-cap vials were used and 9.4 ml of "MF" (Merthiolate-formalin) solution was added prior to departure from Cairo. Lugol's iodine solution (0.6 ml) was added to the MF solution just prior to addition of feces to each vial. Samples having the size of a garden pea were selected from three different parts of each stool. The fresh fecal material was mixed thoroughly with MIF solution with

[1] The opinions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views of the Navy Department.

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the aid of an applicator stick. A separate sample of feces was fixed in a 15-ml vial containing 8 per cent formalin.

After returning to Cairo all vials were sealed by dipping the upper part of vial and cap into a heated mixture of paraffin and wax. Stool specimens were examined after return of the senior author to the United States. Three preparations (22 x 22 mm coverslip) of several drops each (one preparation by each of three different examiners) were examined from the MIF fixed material, withdrawing the samples by pipette from the uppermost layer of the particulate sediment. The formalin-fixed samples were subjected to the AMS-III technic of Hunter, *et al.*, (1948) for the concentration of helminth eggs.

SURVEY FINDINGS

Usually it is difficult or almost impossible to obtain an accurate parasite-incidence picture, especially for the protozoa, from materials collected on a hurried trip through a new or foreign area. The use of the simple but reliable MIF preservation technic, however, has permitted us to make a reasonably accurate study of both the intestinal protozoa and helminths from sizable samples 2 years after the date of collection. Table 1 gives the pertinent information on such materials taken in Turkey.

The incidence of protozoa is not as high as might be expected in this part of the world where the standards of sanitation are only mediocre. General customs of the population tend to encourage parasite propagation and there are sufficient numbers of flies to suggest an effective potential of transmission by food contamination. The incidence of *Entamoeba histolytica* reported here, however, is much greater than that given by Wells (1956) employing the MIF preservation method in a study of intestinal parasites in the southern part of Turkey. At the same time he listed 68 per cent of the people as having *Entamoeba coli*, a figure considerably higher than ours. Except for the incidence of small race *E. histolytica* at Istanbul, the greater proportions of the small to the large race *E. histolytica* are similar to those we have recorded in surveys in the Sudan (1955) and Egypt (1956). As in the latter surveys the presence of *Entamoeba coli*, in general, is similar to the total incidence of *E. histolytica*.

The incidence of the other protozoa is not unusual except for the fact that only one *Isospora* was found among the 350 stools studied and the rate for *Giardia* at Sapanca is rather high for adults. An incidence of 8 per cent for *Dientamoeba fragilis* reflects the effectiveness of the preservation of delicate organisms by the MIF method. Higher figures in the entire column for the Sapanca area are none too surprising since observations on the spot suggested a much greater parasite transmission potential than at the other sites of fecal collection.

It should, perhaps, be pointed out that the data on incidence presented here may not necessarily be representative of the incidence in the population at large. The donors were military personnel who had access to medical care at military hospitals, thus increasing their chances of having been subjected to treatment with antibiotics and/or drugs unfavorable for the well-being of intestinal protozoa.

Although the incidence of the helminths, in general, is not unusual, a total of 19 species is listed. The infection rates for *Ascaris* and *Trichuris* are as was expected, but the rate of infection with *Trichostrongylus* seems a little low since many people in the general population were closely associated with pets and larger domestic animals. The latter condition, or close relationship of man with animals, was especially evident at Sapanca from which the greater number of infections was noted. Rates of helminth detection were increased in a number of instances as a result of employing the sodium sulfate, hydrochloric acid and Triton (AMS-III) technic. In the case of *Trichostrongylus* from Sapanca the increase was 7-fold. Consumption of considerable quantities of fresh vegetables (lettuce, radishes, onions, etc.) by inhabitants of Sapanca at this season of the year was obvious and the majority of stools contained partially digested vegetative matter supporting the expected occurrence of *Heterodera*. Over half of the stools contained unidentified bodies closely resembling in size, structure and color the blastocysts of a freshwater bryozoan.

Two of the 350 specimens contained the eggs of *Hymenolepis diminuta*. A check of individual records revealed that over half of the 13 persons infected with *Diphyllobothrium latum* had come from villages along or near the southern shores of the Black Sea. There is no explanation for

TABLE 1

Incidence (per cent) of intestinal protozoa and helminths in peoples of western (Anatolia) Turkey

Locality	Istanbul	Sapance	Bolu	Ankara
Number of stools examined	50	133	66	100
Protozoa				
Entamoeba histolytica (large race)*	12†	4	5	5
Entamoeba histolytica (small race)	4	22	14	6
E. histolytica (l. & s. races together)	2	16	5	4
Total *E. histolytica*	18	42	24	15
Entamoeba coli	10	34	24	15
Endolimax nana	2	36	26	17
Dientamoeba fragilis	—	8	3	1
Iodamoeba bütschlii	12	20	15	15
Chilomastix mesnili	—	4	2	2
Entermonas hominis	8	22	15	8
Giardia lamblia	10	21	16	12
Trichomonas hominis	—	2	1	2
Isospora belli	2	—	—	—
Helminths				
Ascaris lumbricoides	30/26‡	43/42	36/36	26/24
Enterobius vermicularis	6/2	2/-	3/6	-/2
Heterodera sp.	-/-	14/1	-/-	-/-
Hookworm	4/8	8/13	2/6	4/9
Strongyloides stercoralis	2/-	1/-	-/-	1/-
Trichostrongylus sp.	-/-	2/15	2/1	-/-
Trichuris trichiura	16/48	20/45	12/24	14/45
Diphyllobothrium latum	4/2	4/1	2/2	3/2
Hymenolepis diminuta	-/-	-/1	-/-	-/1
Hymenolepis nana	-/4	-/-	-/-	2/2
Taenia sp.	4/4	8/7	3/3	1/2
Dicrocoelium dendriticum	14/44	4/2	3/7	6/43
Fasciola sp.	2/2	-/1	-/2	1/0
Fasciolopsis buski	-/-	-/-	-/1	-/-
Clonorchis sinensis	-/-	-/-	-/1	-/-
Heterophyes heterophyes	4/4	2/2	-/1	-/-
Heterophyes taichui	-/2	1/1	-/-	-/-
Opisthorchis felineus	-/2	-/1	-/-	-/-
Schistosoma mansoni	-/2	-/2	-/-	-/-
Tyroglyphoid mite	-/2	8/2	-/-	-/-
Specimens with no protozoa	10	14	16	12
Specimens with no helminths	20/14	16/13	12/14	17/10
Specimens with neither protozoa or helminths	8	5	7	8

* Large race: cysts measuring more than 10μ; small race: less than 10μ.

† Values given as nearest whole number.

‡ Incidence of helminth eggs based upon examination of formalin-fixed stools subjected to the AMS-III concentration method.

the low incidence of *Hymenolepis nana* and especially its absence at Sapanca where general conditions would seem to favor transmission of this parasite. On the other hand, Wells (1956) did not report this cestode from his survey in southern Turkey.

Information gained from the finding of eight species of trematodes reveals several points of epidemiologic interest. Our records, which included information on unusual activities, travel and residence during the period of 3 years prior to this survey, indicate that "he who travels or fights abroad" may return home with parasites uncommon to his homeland. This apparently is the case with two soldiers in a small detachment at Bolu. One passed the eggs of *Clonorchis sinensis*, the other of *Fasciolopsis buski*. Questioning at the time of collection of fecal specimens revealed that both men had spent time with the United Nations forces in Korea. Travel was also responsible for the infection of at least two of the three persons with *Schistosoma mansoni*. These two stated that they had travelled to Mecca with the annual Moslem pilgrimage and one recalled that he had bathed in a small open stream while in Arabia.

Dicrocoelium eggs occurred in more than 40 per cent of stools from Istanbul and Ankara which were subjected to the AMS-III concentration technic. Although in recent years *Dicrocoelium* has been reported with greater frequency in the peoples of eastern Europe and western Asia, it seems that the majority of these are spurious cases. A number of eggs appeared to be nonfertile or immature when fixed, suggesting digestion of adult worms and subsequent passage of eggs through the intestinal tract after incidental consumption with raw or poorly-cooked sheep livers. Others, however, appeared to be normal, viable eggs, possibly representing genuine infections. A similar situation exists relating to questionable infections with *Fasciola*. Large numbers of *Dicrocoelium* were found in sheep examined from abbatoirs at Istanbul and Ankara, and *Fasciola hepatica* and *F. gigantica* were found in domestic stock at Istanbul, Sapanca and Bolu. Neither *Dicrocoelium* nor *Fasciola* was listed from southern Turkey by Wells (1956).

Eggs of *Heterophyes heterophyes* and *H. taichui* were identical with those observed by us in stool specimens from the people residing in Lower Egypt along the southern shores of the Mediterranean. The presence of eggs of *Opisthorchis felineus* in one person from Istanbul and another from Sapanca probably represents accidental infections.

There was an absence of intestinal protozoa and/or helminths in only a few persons. Some of these had been recently hospitalized, several for hookworm treatment, and others were known to have been treated with one or more of the antibiotics. The use of the AMS-III concentration method did not greatly alter the negative findings for helminths. This is to be expected, however, since the MIF technic as employed by us, is actually more than a direct smear and there is considerable concentration of intestinal protozoa and helminth eggs by the sedimentation principle.

SUMMARY

An examination of 349 fecal specimens fixed by the MIF (Merthiolate-iodine-formalin) preservation method revealed the presence of ten species of protozoa, seven of nematodes, four of cestodes, eight species of trematodes and a tyroglyphoid mite. There was a predominance of the small race of *Entamoeba histolytica* over the large race and only a single infection with *Isospora* was noted.

Although there was an unusually high incidence of eggs of *Dicrocoelium* the conditions of these eggs in stool specimens indicate spurious infections. *Fasciolopsis* and *Clonorchis* in Turkish soldiers from the Korean campaign implicate travel as the probable reason for infection; *Schistosoma mansoni* infections probably were contracted as a result of pilgrimages to Mecca.

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STUDIES ON THE GROWTH REQUIREMENTS OF *ENTAMOEBA HISTOLYTICA*

The Ingestion of Altered Bacterial Structures by *E. Histolytica* in the Shaffer-Frye Medium[1]

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Since the original description of the Shaffer-Frye (S-F) medium (Shaffer, Ryden and Frye, 1948) numerous studies have been made in an effort to determine the critical growth factors in the medium, that are responsible for propagation of *Entamoeba histolytica*.

The S-F medium is prepared as follows: An as-yet-unidentified, Gram-negative, anaerobic streptobacillus[2] is incubated for 24 hours at 37°C in fluid thioglycollate medium (Baltimore Biological Laboratories No. 136C) containing 1.0 per cent glucose and 1.0 per cent rice starch. This culture is then centrifuged horizontally (International PR2) at 2,000 rpm for 45 minutes. The supernatant fluid, which is known to contain 30 to 70 million viable bacterial cells per ml, is collected and used as the base for the medium. Of this fluid, 2.5 ml are added to culture tubes containing 0.1 ml of normal horse serum and 0.7 ml of saline (0.85 per cent NaCl) which contains 1,000 units of penicillin G potassium. The medium is then inoculated with appropriate material containing inocula of *E. histolytica* and layered with petrolatum to maintain anaerobic conditions. The amebas propagate very actively in the medium and have characteristic growth curves depending on the strain of *E. histolytica* being used.

Originally it was thought that the bacterial propagation in the fluid thioglycollate medium in some way "preconditioned" this medium by means of (1) some by-product or by-products of metabolism, (2) the production of some set of physical conditions suitable for propagation of *E. histolytica*, or (3) a combination of both of the above (Shaffer, Ryden and Frye, 1948, 1949). However, later evidence indicated that it was not a matter of "preconditioning" the medium, but rather a question of providing an appropriate number of suitable bacterial cells for propagation of the amebas (Shaffer, 1952, 1953). This evidence was supplied by the observation that (1) bacterial cells suspended in fresh, fluid, thioglycollate medium could be used in making up the S-F medium and that (2) the amount of propagation of *E. histolytica* in S-F medium was directly related to the number of bacterial cells present in the medium at the time it was made up. It was furthermore observed that the decrease in viable bacterial cells in the S-F medium was greater in the presence of propagating *E. histolytica* than it was when only the penicillin G potassium was present. This suggested that the amebas were either destroying bacterial cells or competing with them for some substance or substances necessary for their survival. Whatever the explanation might be, it appeared that the vital material for amebic propagation was closely associated with the bacterial cell.

These observations present at least two questions of fundamental importance in the propagation of *E. histolytica* in the S-F medium. One of these questions involves the means by which the ameba gets what it needs from the bacterial cell and the other question, and probably the most important one, involves the nature of this material.

The experiments to be described in this report were designed to study possible means by which the amebas obtain the material they need from the bacterial cell.

There are at least two possible ways by which *E. histolytica* might obtain material from the bacterial cells. First, the bacterial cells might be ingested by the trophozoites of *E. histolytica* and either digested or the critical material absorbed from the cell and, second, the trophozoites might absorb material from the bacterial cell, without ingestion, merely by making a close contact. Of the two possibilities, the first seemed most reason-

[1] This investigation was supported by a research grant from the National Microbiological Institute (E-499C3) of the National Institutes of Health, Public Health Service, U. S. Dept. of Health, Education and Welfare.

[2] On the basis of morphological and cultural characteristics, the streptobacillus is believed to belong to the genus *Bacteroides*.

able and likely to lend itself most readily to investigation.

The first attempts to demonstrate ingested bacterial cells in the *E. histolytica* trophozoites in S-F medium utilized various fixation and staining techniques. Trophozoites, concentrated from cultures 3 to 48 hours old, were fixed using numerous fixatives, including methyl alcohol, Bouin's fluid, Schaudinn's fixative, 10% formalin, etc., and stained in several different ways. Although structures were occasionally seen in the trophozoites which might have been bacteria, it was impossible to be sure that they were not normal structures or artifacts resulting from fixation. Following this, attempts were made to stain the trophozoites prior to fixation by adding the stains to concentrated suspensions of the amebas. Many variations of stains and techniques were tried, but no definite demonstration of bacterial cells inside the amebas was ever accomplished. Negative results on the procedures used could not be considered definitive.

There remained the possibility that direct microscopic examination of the amebas might prove definitive and it was considered that, of the methods directly available, darkfield or phase microscopy might be the best. Preliminary observations indicated that the internal structure of the *E. histolytica* trophozoites from S-F medium could easily be observed in this manner. Since the darkfield was most readily available, it was chosen for the observations to be described.

As a preliminary to more definitive observations on the amebic trophozoites, it was necessary to become thoroughly familiar with the appearance of the bacterial cells in the S-F medium during the course of incubation at 37°C as seen in darkfield preparations. The technique for studying the bacterial cells was as follows: The S-F medium was made up in the usual manner, but was not inoculated with *E. histolytica*. After layering with petrolatum the tubes were incubated at 37°C and removed for examination at specified intervals varying from 3 to 48 hours. For examination, the tubes were placed in the International PR2 centrifuge and spun at 1,500 rpm for 30 minutes, after which all but 0.5 ml of the supernatant fluid was removed and the tube shaken to suspend the sediment. A drop of this suspension was placed on a slide and sealed under a coverslip with petrolatum. Examination was made with a Spencer darkfield microscope.

The results of these observations were of considerable interest. Certain changes occurred in the morphology of the bacterial cells, presumably as a result of the action of the penicillin G present in the medium. Some of the cells exhibited an uneven cell division resulting in the production of some very short forms. These cells showed a peculiar type of movement, taking the form of constant bending and rotation of the short portion of the dividing cell during the division process. Another change, seen very infrequently in the first few hours of incubation, but with increasing frequency after 10 to 12 hours, involved the appearance of a small, bubble-like structure on the side of the bacterial cell. These structures enlarged until the resulting round body had a diameter about half the length of the bacterial cell. Occasionally, more than one appeared on a single bacterial cell. As time went on these bodies appeared free in the fluid and their number increased until, after 24 hours, they predominated and there were few typical bacillary cells remaining. At this point the size of these bodies varied from very small, granule-size bodies to structures of the size described above. It has not yet been determined what the fate of the bacterial cell is in these preparations. These changes resemble those described by Hahn and Ciak (1957) in cells of a strain of *Escherichia coli* when exposed to penicillin.

Stained smears made from the suspensions used for darkfield examination confirmed these observations as indicated by the photomicrographs shown in Figure 1.

The next step was to study cultures of *E. histolytica* in the S-F medium by darkfield microscopy to determine whether or not any of the structures described above could be seen inside the trophozoites during the propagation of the amebas. Two strains of *E. histolytica* were used throughout for these observations, strain K-9[3] and strain 103.[4] Both behaved in the same manner. Preliminary observations were made as follows: Cultures of *E. histolytica* were made up in the usual manner and incubated at 37°C. At specified times, varying from 3 to 48 hours, tubes

[3] The K-9 strain was obtained from Dr. M. M. Brooke of the Communicable Disease Center, Public Health Service. It was brought from Korea in 1951 by Dr. W. W. Frye.

[4] The 103 strain was obtained from Mr. M. C. McCowen of the Lilly Research Laboratories. Mr. McCowen had obtained the culture from Dr. C. W. Rees of the National Institutes of Health.

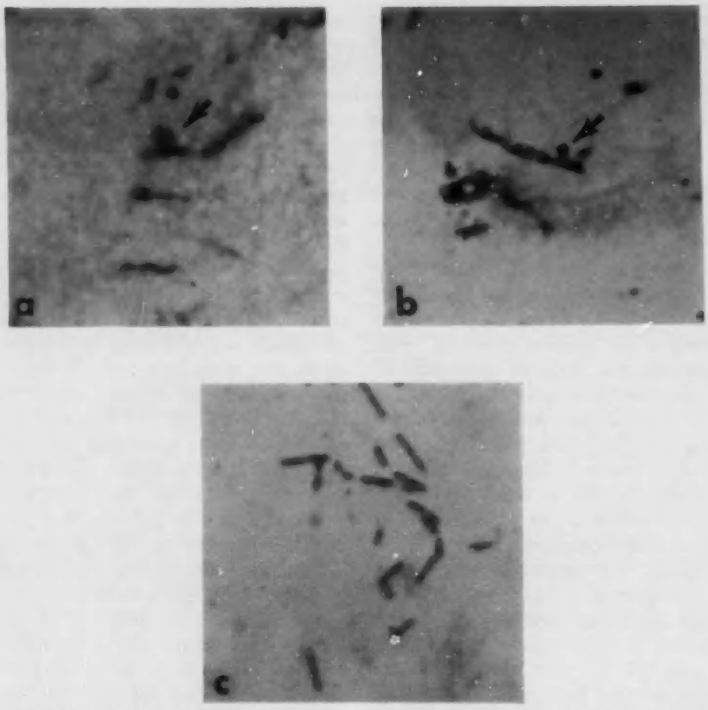

FIG. 1. Round bodies formed by the Gram-negative, anaerobic streptobacillus under the influence of penicillin G. Bacillary body is thickened and granular with round body attached to the lateral surface of the cell. (a) Arrow indicates large round body evidently well advanced. (b) Arrow indicates small, densely stained body, typical of early stage in development. (c) Normal culture of streptobacillus untreated with penicillin G. Wright's stain × 2000.

were removed and samples were taken from the bottom of the cultures by means of a capillary pipette and placed on a slide. This was sealed under a coverslip with petrolatum and examined with the darkfield microscope.

At no time were any typical bacterial cells seen inside the amebic trophozoites. However, it was noted frequently that bacteria were in close contact with the cell wall of the amebas where they might remain for brief preiods and then float free in the fluid again.

The amebic trophozoites exhibited cytoplasmic inclusions. These were round and very few in number in the early cultures, but their numbers increased with time. Morphologically, these inclusions appeared to be identical with the round bodies previously described, which were derived from the bacterial cells. The number of inclusions in the amebas roughly paralleled the number of round bodies present in the culture fluid. Continuous observation of single amebas showed that they were indeed ingesting these bodies and the process was observed repeatedly. The penicillin G appeared to be bringing about the production of large numbers of the round bodies, which in turn were ingested by the amebas. It still remains to be shown whether or not these bodies supply the material necessary for continued propagation of *E. histolytica* in S-F medium. The observations to be presented next constitute circumstantial evidence in support of this idea.

There have been for some time in this laboratory six cultures of the Gram-negative, anaerobic streptobacillus. Culturally and morphologically these cultures appear to be identical. The only difference between the six cultures, recognized at the time these experiments were initiated, was in

their ability to support the propagation of *E. histolytica* when used to make the S-F medium. If culture No. 1 was used for S-F medium, the amebas propagated very actively. With culture No. 2, propagation was somewhat less; with cultures 3 and 4, the amebas did not propagate quite as well as with No. 2; and with cultures 5 and 6 the amebas failed to propagate. It is probably most appropriate to term these cultures "variants" since they were all derived from the original Gram-negative, anaerobic streptobacillus isolated from the mixed bacterial flora of the NRS strain of *E. histolytica* (Shaffer, Walton and Frye, 1948).

The observations on the round bodies described previously were made with culture No. 1, which was currently being used for routine maintenance of *E. histolytica* in the S-F medium. It was of interest to make similar observations on the other five "variants" and attempt, if possible, to correlate the production of round bodies under the influence of penicillin G with the ability of the "variants" to support propagation of *E. histolytica* in S-F medium.

The S-F medium was made in the routine manner from supernatant fluids of each of the six "variants". An appropriate number of tubes made from each culture was inoculated with the K-9 strain of *E. histolytica* and incubated at 37°C. At the end of 12, 24 and 48 hours, the relative numbers of round bodies in randomly selected tubes from each variant were determined by darkfield microscopy and recorded as average number per field. At the end of 24 and 48 hours the amount of propagation of *E. histolytica* was estimated by direct microscopic observation of the cultures (Shaffer, Ryden and Frye, 1948). Previous experiments have shown that such observations parallel counts done with the counting chamber.

Table 1 shows the composite results of several experiments on the six "variants" done in the manner described above.

The results shown in Table 1 reveal some evidence of relationships between the production of round bodies and propagation of the K-9 strain of *E. histolytica*. Only the first two transfers of *E. histolytica* are shown in the table, since subsequent transfers were all consistent with these. Bacterial culture No. 1 produced the greatest number of round bodies, and the propagation of the amebas was most rapid and consistent in these cultures.

TABLE 1

Relationship between round body production and ability to support propagation of Entamoeba histolytica *by six "variants" of the Gram-negative, anaerobic streptobacillus in the S-F Medium*

Culture no.	Average no. of "round bodies" per microscopic field			Propagation of *E. histolytica**			
				1st transfer		2nd transfer	
	12 hr	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
1	2.5	7.0	8.2	4+†	4+	4+	4+
2	0.5	4.5	4.5	1+	4+	2+	4+
3	1.8	3.6	6.3	3+	4+	2+	3+
4	1.7	3.1	4.3	1+	3+	1+	2+
5	0.2	0.1	0.5	3+	2+	1+	0
6	0.1	0.0	0.2	±	0	0	0

* The K-9 strain of *E. histolytica* was used throughout these experiments.

† Readings: 4+ = 25 or more amebas/low-power microscope field; 3+ = 15–24 amebas/low-power field; 2+ = 5–14 amebas/low-power field; 1+ = 1–4 amebas/low-power field; ± = less than one ameba/low-power field.

Fewer round bodies were present in culture No. 2 and propagation of the amebas was slower, but nevertheless reached 4+ after 48 hours. Culture No. 3 had a considerable number of round bodies after 48 hours but less at 24 hours and propagation of the amebas was not as consistent as it was with cultures No. 1 and No. 2. With culture No. 4, round body production was somewhat slower than in cultures No. 1, No. 2 and No. 3, and propagation of the amebas was considerably less. Some propagation or survival of the amebas appeared to occur on the first transfer in culture No. 5 but not on the second transfer; with culture No. 6 no propagation of amebas could be detected. Round body production in cultures No. 5 and No. 6 was very low. Thus, these results are suggestive of some relation between round body production and propagation of the K-9 strain of *E. histolytica*. It should be pointed out that five other strains of *E. histolytica* have been observed to behave similarly in S-F medium made from these six variants of the streptobacillus.

Further investigation of the conditions under which the round bodies are formed by the streptobacillus have revealed that: (1) they do not form in the absence of penicillin G; (2) they form with or without normal horse serum, but their formation seems to be enhanced in its presence pro-

TABLE 2

Results of penicillin sensitivity test on six "variants" of the Gram-negative, anaerobic streptobacillus

Penicillin concentration	Growth of bacteria after 24 and 48 hours in culture number											
	1		2		3		4		5		6	
	24	48	24	48	24	48	24	48	24	48	24	48
0.5	+*	+	+	++	+	++	+	++	+	++	+	+++
1.0	±	+	±	+	−	+	+	++	+	++	+	+++
3.0	−	+	±	+	−	+	+	+	+	++	+	+++
5.0	−	+	−	+	−	+	−	±	+	++	+	+++
7.5	−	−	−	−	−	±	−	±	−	±	+	++
10.0	−	−	−	−	−	−	−	−	−	±	+	+
20.0	−	−	−	−	−	−	−	−	−	−	−	±
50.0	−	−	−	−	−	−	−	−	−	−	−	−
0.0	+	++	+	++	±	++	+	++	+	++	+	++

* Visible growth of streptobacillus.

vided the penicillin G is present; (3) the presence (up to 1.0 per cent) or absence of glucose has no effect on the formation of round bodies; and (4) the formation of the round bodies appears to begin earlier in the presence of actively propagating *E. histolytica* than in tubes without amebas.

Since penicillin G appears, at the present time, to be of critical importance, it was of interest to compare the penicillin sensitivity of the six variants of the streptobacillus discussed before. Accordingly, standard inocula of the six variants were introduced into tubes of fluid thioglycollate medium containing varying concentrations of penicillin G and incubated at 37°C. The results of this experiment are shown in Table 2.

The results shown in Table 2 reveal that considerable difference exists in the sensitivity of these six variants to penicillin G. It is considered likely that the 24-hour readings are of greater significance than the 48-hour readings since Na thioglycollate is known to inactivate penicillin. Culture No. 1 was completely inhibited by 3.0 units, and partially inhibited by 1.0 unit, of penicillin G at 24 hours, while culture No. 2 was partially inhibited by 1.0 and 3.0 units and completely inhibited by 5.0 units. The results with culture No. 3 are hard to interpret since it grew more slowly than the others, but judging from the 48-hour reading, its sensitivity was possibly less than that of either No. 1 or No. 2. Culture No. 4 grew in the presence of 3.0 units but not in the presence of 5.0 units during the first 24 hours. With cultures No. 5 and No. 6, there appears definite evidence of more resistance to the action of penicillin G.

DISCUSSION

The exact conditions necessary for propagation of *E. histolytica* are, as yet, unknown. It is obvious that physical conditions such as pH, oxidation-reduction potential, temperature, etc., are of great importance, but these can be produced artificially without much difficulty. The most critical factors appear to be the material or materials supplied by the cells (bacteria, trypanosomes or tissue cells) with which the amebas grow in culture or, indeed, in the host.

One of the characteristics of *E. histolytica* in the infected human host is its failure to ingest bacteria to any considerable extent, although there are conflicting views on this (Reichenow, 1937; Dobell, 1919; Hoare, 1952). *E. histolytica* ingests red blood cells quite regularly, but there is no in vitro evidence that red blood cells supply any critical growth materials.

In the trypanosome medium of Phillips (1951), ingestion of trypanosomes by the amebas is regularly observed. Where propagated in tissue-bearing cultures (Shaffer, *et al.*, 1953) no evidence of ingestion of tissue cells or fragments was observed. Thus, the ingestion of recognizable particulate material would appear to be irregular in the various media.

The observation on round body production by the streptobacillus under the influence of penicillin G and its possible relationship to the propaga-

tion of *E. histolytica* in the S-F medium, presents suggestive evidence of a relation between ingestion of particles and growth and multiplication of these amebas. It is possible that altered tissue or bacterial structures are ingested by the amebas in other substrates. Such an occurrence could easily be overlooked in mixed bacterial flora, tissue-bearing substrate or monobacterial cultures of *E. histolytica*. It would be of interest to know whether or not ingestion of particulate cellular elements (mitochondria?) occurs in the cultures described by Baernstein, Rees and Bartgis (1957) in which they obtained propagation of *E. histolytica* in apparently cell-free supernates of chick-embryo-tissue homogenates.

Some workers have reported that the incorporation of penicillin and streptomycin in cultures of *E. histolytica* being maintained in the laboratory in mixed bacterial flora or for primary isolation from stool material, enhances the propagation of the amebas. It has been assumed that the function of the antibiotics in such cultures was to prevent excessive propagation of the accompanying bacteria. Although this inhibition of bacterial multiplication is probably of the greatest importance, it is possible that the effect of penicillin on certain of the bacterial cells may have some enhancing effect on the propagation of the amebas.

The results shown in Table 1 indicate a rough, quantitative relationship between the propagation of *E. histolytica* and the production of round bodies by the streptobacillus cultures. Technical problems have made it difficult to follow the quantitative relationship between round bodies and amebas through the entire growth cycle of the culture. That the number of round bodies and the propagation of the amebas is not always parallel is indicated by the occasional observation of large numbers of round bodies in cultures 48 to 72 hours old, at which time the ameba population is beginning to decrease rapidly (Shaffer, 1952). Such observations may indicate that: (1) there may be a stage in the development of the round body which is useful to the amebas and, once the body has progressed to a certain point, it is no longer of any use; (2) the round bodies seen in the older cultures may already have given up what the amebas need; (3) other, unrecognized factors make utilization of the round bodies impossible; (4) the round bodies may be completely unimportant in the propagation of *E. histolytica*. Experiments are now in progress which it is hoped will provide answers to some of the above points. Chemical analyses of bacterial sediments and S-F medium with and without penicillin G are being made and have yielded results showing differences between the streptobacilli that do and do not support propagation of *E. histolytica*. These will be reported at an early date (Goldfarb, et al., In preparation).

SUMMARY

The formation of round bodies by the anaerobic, Gram-negative streptobacillus used in the S-F medium under the influence of penicillin G has been reported.

Actively propagating *E. histolytica* trophozoites ingest the round bodies roughly in proportion to the numbers available.

Streptobacillus variants that do not support propagation of *E. histolytica* do not produce the round bodies. There is a rough parallel between round body production and ability to support propagation of *E. histolytica* in six variants studied.

Normal horse serum and glucose do not influence the production of the round bodies.

Those variants not producing round bodies in the presence of penicillin G are somewhat more resistant to the antibiotic than are those which produce round bodies.

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WINTER AND SPRING SURVIVAL OF *AEDES AEGYPTI* IN SOUTHWESTERN GEORGIA

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INTRODUCTION

In October, 1956, a survey was conducted in Thomasville, Georgia to determine the extent of *Aedes aegypti* breeding. This mosquito was found in 85 per cent of 88 square blocks of the city inspected and at 41.8 per cent of the premises examined.

Thomasville has a population of approximately 15,000 and is located 12 miles north of the Florida state line and 55 miles east of the Alabama line. Included in the survey were representative blocks of business and industrial areas and various classes of residential areas. No inspections were made within houses or business establishments. The premises index of 41.8 was one of the highest reported from 23 cities in the United States surveyed for *Ae. aegypti* by the Communicable Disease Center in 1956. Since large numbers of larvae were present, a study of the winter ecology of *Ae. aegypti* was initiated in November, 1956, in an effort to provide information of possible value to any subsequent attempts to control or eradicate the species in the United States.

METHODS

Weekly observations starting on November 12, 1956, were planned to determine the effects of winter temperatures and precipitation on the survival and development of larvae in (1) various types of semi-permanent containers located in an experimental plot, (2) in similar containers located throughout the city, and (3) in incidental containers at premises in lower-class residential areas.

Experimental plot. Five each of tin cans, wide mouth jars, 5-gallon buckets, and old tires which were breeding *Ae. aegypti* larvae were placed 3 to 4 feet apart in a fenced, 20-by-30-foot experimental plot. An additional, control, container of each type, known not to be infested with eggs or larvae, was partially filled with water and also placed in the experimental plot. This plot was located between one of the lower-class residential areas and an industrial area. It was shaded by trees and a low embankment on the east side while the other sides were exposed to the full effects of natural conditions. A continuous-recording thermograph which charted temperatures at ground level and 3 feet above the ground was also located within the plot. Daily rainfall records were obtained from a U. S. Weather Bureau station located about three blocks from the experimental plot.

Semi-permanent containers in different areas of the city. Groups of five each of 55-gallon metal drums, 5-gallon buckets, cemetery urns, wide-mouth jars, tin cans, and tires which were producing *Ae. aegypti* at the beginning of observations were located in industrial and residential areas and were inspected weekly as in the experimental plot.

Incidental containers in low-class residential areas. Fifteen to 20 premises representative of the area were inspected weekly for any containers which could possibly produce or harbor *Ae. aegypti* larvae. The containers most commonly inspected were small bottles, animal drinking pans, wide-mouth jars, tin cans, tubs, wash pots, and old tires.

The presence of mosquito larvae was determined weekly by visual inspection of wide-mouth glass jars, by dipping in the smaller containers with a shallow soup ladle, and by dipping in the larger, deep containers with white-enamelled water dippers. Samples of the larvae obtained by dipping were identified in the laboratory if any doubt existed as to their identity. A complete examination of the contents of each container in the experimental plot was made on April 8, 1957, and samples of all mosquito larvae present were identified by microscopic examination. The final inspection was made on June 17. Due to inclement weather conditions no weekly inspection was made on April 1, 1957, and one residential inspection was omitted during the week of December 24, 1956.

Average weekly temperatures were computed

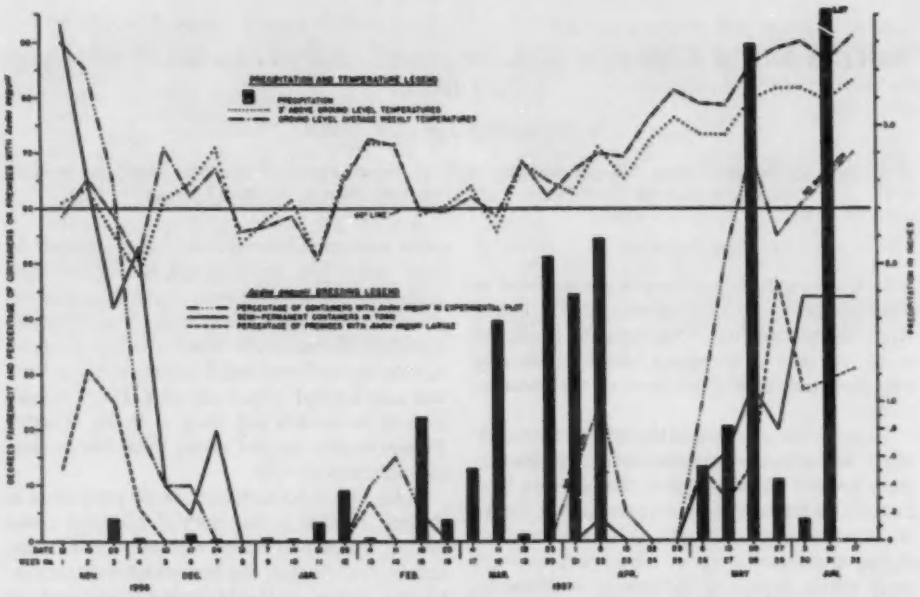

FIG. 1. Weekly precipitation, average weekly temperatures at ground level and three feet above ground level, and the occurrence of *Aedes aegypti* breeding at Thomasville, Thomas County, Georgia, November, 1956 to June, 1957.

by dividing the sum of the daily high and low temperatures by 14. Weekly precipitation was the total recorded from 7 a.m., the Monday of inspection to 7 a.m., the succeeding Monday.

RESULTS AND DISCUSSION

On November 5, 1956, all of the containers except one control of each type located in the experimental plot and all of the semi-permanent containers, except one 55-gallon drum, located throughout the city contained *Ae. aegypti* larvae. No inspection of incidental containers at residences was made at this time. The findings at weekly periods thereafter as determined by dipping and visual inspection may be summarized as follows.

Experimental plot. At the end of week 1 (November 12), 90 per cent of the experimental containers had *Ae. aegypti* larvae, as shown in Figure 1. During week 2, the average ground temperature rose from 58.5° to 65° F. This rise was accompanied by a decline from 90 to 85 per cent in containers positive for *Ae. aegypti*, probably as a result of emergence of adults. During weeks 3 and 4, average weekly ground temperatures declined sharply from 65° to 48° F. The percentage of containers with larvae declined from 85 to 20, many larvae appearing weak or dead and, although average ground temperature rose to 71° F during the next week, the percentage of containers with larvae continued to decline to 10 per cent. By the end of week 7 (December 24), no larvae could be seen or were dipped from the containers although average weekly ground-level temperatures were above 62° F. No larvae were found again for a 5-week period. Average ground-level temperatures were below 60° F during most of these weeks but rose abruptly to 64.5° and 73.5° F, respectively, during the last week of the period. These rises apparently activated larvae so that fourth-instar larvae were taken in 10, 15, and 5 per cent of the containers during the subsequent 3 weeks (weeks 13, 14, 15). After the fourteenth week, precipitation, which had been insufficient to maintain the original water levels in the containers, occurred weekly in sufficient quantity to raise the levels in all containers. Average weekly ground-level temperatures remained below 60° F until week 19, and no larvae could be found until

the containers were emptied and their contents thoroughly searched on April 8, 1957 (week 22). At that time it was found that some of the deeper containers, such as 5-gallon buckets and old tires, had a small number of larvae, no more than six being found in any one receptacle. The containers which held larvae were those located in more sheltered positions or in which larvae apparently were afforded protection by large amounts of organic debris. The larvae were in the second, third, and fourth instars. It is doubtful that any of these could have been discovered by routine visual inspection or dipping. The tin cans, wide-mouth glass jars, and control containers had no *Ae. aegypti* larvae. Ground-level temperatures averaged above 65° F during 2 of the 3 weeks preceding the complete inspection on April 8. The containers used as controls produced only *Culex* larvae.

It was concluded that during a relatively mild winter season, with coldest temperatures during the early part, some *Ae. aegypti* larvae survive and may be activated even in midwinter by average weekly ground-level temperatures above 65° F, if such temperatures prevail for 2 weeks or longer. No indications of feeding or oviposition were observed during the colder months. Larvae were present on May 6 and each week thereafter until inspections were terminated on June 17.

The tree-hole breeding species, *Ae. triseriatus*, was first found in tin cans on December 10 (week 5) and continued present in 10 to 40 per cent of all the containers until May 6, after which time it was not seen again.

Culex mosquitoes, chiefly *C. quinquefasciatus* and *C. restuans*, infested 65 per cent of the receptacles at the end of week 1, reached a low infestation of 10 per cent during the coldest weather (week 4), and maintained an average infestation of 53 per cent throughout the observation period. *C. restuans* was predominant during the colder months, reaching its greatest abundance in March. Few larvae of this species were present the first week in May. *C. territans* occurred in the containers during March, April, and May in small numbers.

Semi-permanent containers at various locations. At the end of week 1, 93.3 per cent of the semi-permanent containers scattered throughout the city contained *Ae. aegypti* larvae. During the first 6 weeks, the percentage positive declined much like that of containers in the experimental plot. However, a slight rise during week 4, and a higher rise in week 7, occurred which were not in unison with the general decline noted in the experimental plot. No larvae were found from the eighth through the twentieth week; no inspection was made during the twenty-first week. On the following week, larvae were found only in one of the tires regularly inspected.

Small containers such as concrete and marble urns, tin cans, and wide-mouth glass jars located in a cemetery became dry and lost their infestations of *Ae. aegypti* during the first 7 weeks of the observation period, and no larvae of this species were found in them after that time. Two urns located under an oak tree retained small amounts of water and continued to harbor *Ae. triseriatus* larvae throughout the period. *Culex* were present in 10 to 65 per cent of the containers throughout all the inspections.

Incidental containers in lower class residential areas. Ae. aegypti larvae were found in containers at residences only from November 12 through December 3, 1956, and during week 13 (February 4, 1957). During the latter week, three larvae in the third and fourth instars were found in a tire. No more larvae were located in the residential areas until May 6. In five inspections between May 6 and June 17, the percentages of residences where larvae were found varied between 7 and 47, with old tires the favorite breeding containers.

SUMMARY

In southwestern Georgia observations on the effects of low termperatures on the survival of naturally occurring *Aedes aegypti* larvae in various types of breeding containers were made throughout the winter. Average weekly air temperatures below 60° F killed most larvae but, because of the sheltered positions of some containers, a few survived during periods when the average weekly temperature was as low as 48° F. During warmer parts of the winter, larval development continued and adults emerged. No indications of feeding or oviposition were observed during the colder months. In the area studied, normal breeding and oviposition apparently commenced during May.

PHAGE ABSORBED ON PAPER DISKS IN THE RAPID IDENTIFICATION OF *VIBRIO COMMA*

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It has been shown that certain phages, in particular type A, lyse true, agglutinable cholera vibrios. This test is useful in the identification of *Vibrio comma* (White, 1936; Seal, 1957). The maintenance of phages, however, is laborious and requires constant control. They rapidly lose their activity in media. Thus, phage suspensions are not easy to use for diagnostic purposes. However, Cavanaugh et al., (1953) reported that filter paper strips saturated with *P. pestis* phage remained active for a long time and were helpful in identifying this organism. Bernstein (1957, personal communication) could maintain *Xanthomonas pruni* phage dried on filter paper over 1 year without loss of titer. Thus, it appeared that paper disks saturated with vibrio phage also should be tested and, if feasible, used in the rapid identification of vibrios; especially in small laboratories and in field surveys.

Four phage lysates were selected for this study because of their broad host range against cholera vibrio strains. They differed from each other in cross tests with resistant strains derived by treatment with individual phage lysates from an Ogawa type organism, labeled 154. The four phage lysates were: *Q. 167 pl*, the propagating strain of which was Ogawa 154; phage *P.R. 15*, received from India; *ES-293*, isolated from an old Japanese culture (Hata); and *Inaba-256* from an Indian strain (Calcutta). The latter two strains were found to be lysogenic in this laboratory. The lysates were prepared by the aeration procedure (Adams, 1950).

Filter paper, U. S. Army Stock No. 4-361-000, Qualitative, and blotting paper, used by the same institution, were employed after washing with ether. Disks 4 mm in diameter were prepared with a paper puncher, autoclaved and dried in a hot air oven.

The medium used for phage propagation consisted of: pancreatic digest of casein (Difco) 10 gm; beef extract (Difco) 3 gm; NaCl 5 gm; yeast extract (10 per cent yeast suspension extracted in succession at 37°, 50° and 70° C for 2 hours each, then filtered) 100 ml; distilled water, 900 ml. Calcium chloride, anhydrous, 0.2 gm, was added after the pH of the medium was adjusted to 7.6. Fifteen or 7.5 gm of agar were added before adjusting the pH if a solid or a semisolid medium, respectively, was needed. The medium was autoclaved at 15 lb pressure for 15 min.

For the preparation of the phage lysate by aeration, 100 ml of the broth (in a 500-ml Erlenmeyer flask) were seeded with approximately 10^6 organisms. The inoculum consisted of a broth culture incubated overnight. Each of the lysogenic strains was grown separately.

The flasks were then aerated with filtered air through a glass tube which was led into the liquid, and incubated at 31° to 35° C for appropriate periods (8 to 12 hrs) necessary to reach the maximal titer of each phage. At the end of the aeration the cultures were centrifuged at 2,600 rpm for 30 min and the supernatant drawn through a Chamberland filter.

Double-layer propagation of the phages was carried out according to the plaque-count method of Adams (1950). One-tenth ml of a young broth culture of the respective propagating strain and the same volume of the phage lysate, in logarithmic dilutions, were mixed in 2.5-ml semisolid agar and this mixture was poured on a solid agar plate. The preparation of the agars was described above. After overnight incubation at 37° C, that semisolid agar mixture which showed organisms completely lysed by the highest dilution of the phage was scraped from the underlying solid agar, emulsified, stored overnight at 4° C, then centrifuged and filtered.

The titers of these lysates were approximately 2.0×10^9 plaque-forming particles per ml for *Q. 167 pl.*, 3.3×10^8 for *P.R. 15*, 2.1×10^9 for *ES-293*, and 1.7×10^8 for *Inaba-256*.

Batches of 20 paper disks were soaked with 1 ml of each phage lysate in different dilutions. The same number of disks were treated with 1 ml of a mixed phage containing equal volumes of all four undiluted lysates dispensed into the

quarters of a Felsen plate. Desiccation with $CaCl_2$ and then evacuation of the air with a Stokes Cryochem apparatus were carried out overnight at 0.1 mm Hg pressure. The dried phage disks were put into small, sterile, serum vials and stored at 4° C.

The lytic activity of these phage disks was determined immediately after preparation and rechecked at 4-week intervals. In order to study the optimal conditions for the use of the disk method, the media, the culture method and temperature of incubation were investigated.

Blood-agar medium, prepared from blood agar base (Difco) containing human or sheep blood, and the medium for phage propagation, were compared as possible test media.

In the double-layer method, 0.1 ml of an overnight vibrio broth culture was mixed with 2.5 ml of melted, semisolid agar and this mixture was poured over solid agar in a Petri dish.

When the semisolid layer solidified, a disk with phage was placed on it. The plates were incubated at 31° C and 37° C. The greatest phage activity was observed at 31° C, though at 37° C clear-cut lysis was still visible.

Two seed-culture methods were compared. One consisted of growing the organisms in broth overnight and reinoculating into fresh broth, then, after 4 hr of incubation, mixing 0.1 ml of the young culture with 2.5 ml of the semisolid medium and pouring this on a solid-agar layer. The second method employed an overnight broth culture without reinoculation. No definite difference was noted.

Clear, well-defined lytic zones were observed using the double-layer method and the medium described above for the phage propagation. Blood agar gave less favorable results. Better visible zone formation was observed when the surface of the blood plates was inoculated by swabbing, and the disks moistened with a drop of sterile broth before being dropped on the surface of the plates.

The activity of the disks saturated with diluted phage lysates did not decrease after 4 months. It appeared, therefore, that each phage strain could be maintained by this method except *P.R. 15*, which showed a slight decrease in its activity (Table 1). While phage on both papers remained active for the same time, the lytic zone produced using filter-paper disks was broader than that obtained using blotting-paper disks. This difference may be due to the difference in the phage blocking power, i.e., the competition of the absorbing powers of organisms and disks.

FIG. 1. Zone formation around paper disks saturated with phage. Lower row left: Mixed phage absorbed on filter paper. Lower row right: Mixed phage absorbed on blotting paper. Upper row: Controls.

TABLE 1

Highest dilution of phage absorbed on paper producing lytic zone

Phage absorbed on disk	Test organism and age of preparation			
	Inaba 2 (1 day)	Ogawa 8 (1 day)	Inaba 2 (4 mo.)	Ogawa 8 (4 mo.)
Q 167 pl	10^{-5}	10^{-5}	10^{-5}	10^{-5}
PR 15	10^{-4}	10^{-4}	10^{-3}	10^{-3}
ES 293	10^{-4}	10^{-4}	10^{-4}	10^{-4}
Inaba 256	10^{-5}	10^{-4}	10^{-5}	10^{-4}
Mixed Phage*	+++	+++	+++	+++

* Concentrated.

According to these results, the double-layer method, with filter paper disks saturated with undiluted, mixed phage lysates, is preferred.

To date, 31 Inaba, 42 Ogawa, 5 Hikojima, 22 El Tor (Ogawa subtype) and 5 inagglutinable paracholera vibrios have been tested by this method. There were differences in the zones produced by individual phage disks. Mixed phage absorbed on filter paper, however, gave excellent results. All of the Inaba, 41 of the Ogawa, all of the Hikojima, none of the El Tor and none

of the inagglutinable vibrios showed zones of lysis with these disks.

Since the layer method at 31° C and disks with mixed phages gave better results, the following procedure is recommended:

1. The organism suspected of being *V. comma* is grown overnight in the above-described alkaline medium.

2. Of this growth, 0.1 ml is mixed with 2.5 ml semisolid agar and poured atop solid agar in a Petri dish.

3. A filter-paper disk soaked with mixed phage lysate is dropped on the upper layer after solidification.

4. After incubation at preferably 31° C for 24 hours the plates are observed for lysis.

The filter-paper pads should be prepared in a larger laboratory which is equipped to maintain and manufacture them.

This test is, naturally, of limited value but may serve well, especially in small laboratories seldom concerned with cholera work or manned by inexperienced personnel.

SUMMARY

It was found that *Vibrio comma* phages absorbed on filter paper maintain their lytic property for a long period of time. A mixture of four phages covering a wide range of true cholera vibrios was prepared and absorbed on filter paper disks which were stored for 4 months. These disks proved helpful in the identification of *V. comma*.

ACKNOWLEDGMENT

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TETANUS NEONATORUM

A Report of Thirty Two Cases

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Tetanus takes a heavy toll of the newborn in the undeveloped countries of the world. This mortality is estimated at from 5 to 10 per cent in the Artibonite Valley of Haiti. In this valley, the midwife is usually an elderly, untrained woman who knows nothing about sterile technique or the proper care of the infant. The cord, being cut with anything handy, such as an unsterile machette, knife, scissors, or a broken piece of glass, is then tied with an unsterile piece of string or wire. Charcoal, usually mixed with dirt, is applied to the cut stump and wrapped with leaves or any old piece of cloth. Most of the cases of tetanus never reach a hospital since hospitals are few and far between in the Artibonite Valley.

Thirty-two cases of tetanus neonatorum were admitted to the Hôpital Albert Schweitzer between October 10, 1956, and June 24, 1957, of which 17 were females and 15 were males. There were eight deaths, two on the day of admission, one on the third day, one on the fourth day and the other four on the seventh to the tenth day. The relatives who brought the infants to the hospital told the same story, i.e., the baby has been unable to nurse since yesterday and is having "crises" (Creole for spasms and convulsions).

The symptoms appeared on the fifth to the fourteenth day: in 28 cases on the fifth or sixth day; in the other four on the eleventh to the fourteenth day. One child in the last group expired on the tenth hospital day. The cause of death was unknown. The patient was simply found dead by the night nurse. The chest was clear the preceding day and there had been no elevation of temperature. Consent for autopsy could not be obtained.

Three of the cases had elevated temperatures and one had a bronchopneumonia on admission. One of these died on the third day (not the case of bronchopneumonia). In the other two cases the temperatures returned to normal after hydration.

MANAGEMENT

On admission to the ward the patient was given 32 mg of Na phenobarbital intramuscularly and a cutdown was done on the saphenous vein after immobilizing the leg with a splint. A polyethylene tube fitting a 20-gauge needle was inserted into the vein and connected to an infusion bottle. A spinal tap was done to rule out meningitis, a procedure that is of doubtful value and has since been discontinued. One case of fulminating meningococcal meningitis had an admission diagnosis of tetanus until the spinal tap was done. However, in this case there was a high polymorphonuclear blood count, an elevated temperature and a bulging fontanel.

The fluid requirements were calculated for a 24-hour period based on 150 ml per kg and the intravenous drip regulated so that the total amount of fluid would last for the full period. To the fluid was added 20,000 to 40,000 units of tetanus antitoxin and 10 ml of 10 per cent Ca gluconate. This procedure proved disastrous in one case, as a massive sloughing developed on the medial aspect of the lower leg and later required plastic surgery. Chlorpromazine (Largactil, Thorazine) in dosage based on 1 mg per kg every 4 hours, was also added to the infusion. Nothing was given by mouth for the first 4 to 6 days, the mouth being cleaned by wet applicators as necessary.

The umbilicus was cleansed with H_2O_2 daily for the first 3 to 4 days and the dressing kept moist with the same solution. Thirty thousand units of tetanus antitoxin were then injected around the stump. The procedure of keeping the umbilicus moist with H_2O_2 after the initial cleaning is of questionable value.

Sodium phenobarbital, 32 mg every 6 hours as necessary, was used as additional sedation. The patient was turned every 2 hours, a procedure that is necessary to prevent a complicating pneumonia. Procaine penicillin, 500,000 units, was given intramuscularly daily for 5 days and 10,000 units of tetanus antitoxin

intramuscularly daily for 3 days. The total amount of antitoxin given varied from 100,000 to 120,000 units except in the first two cases in which 70,000 and 80,000 units, respectively were given. The polyethylene tubing was removed after 72 to 96 hours and a new cutdown done on the other leg. After the first 24 hours the only addition to the fluids was chlorpromazine. The patients were fed orally or by gavage (5 cases) after the sixth to eighth day.

The complicating pneumonia was treated with Combiotic 2 ml intramuscularly, daily, until the temperature returned to normal and the chest became clear.

DISCUSSION

The average length of stay in the hospital was 41 days, varying from 16 to 62 days. The patients continued to have some degree of convulsions for an average of 7 days. The longest period of convulsions for those who survived was 20 days; this being in a case that was oversedated, receiving 2 grains of Na phenobarbital intramuscularly, and who had to be revived in an oxygen-pressure lock. This case showed no evidence of cerebral damage on clinical examination 6 months later. The shortest period for convulsions was 4 days. Nuclear rigidity lasted from 16 to 32 days. In one case, mentioned above as oversedated, the muscular rigidity lasted 45 days. There were eight cases of bronchopneumonia among the 24 survivals treated with Combiotic. In these oxygen was given as necessary. One case had a necrotic slough and one case a severe, prolonged diarrhea treated with oral tetracycline. The cause of the diarrhea was undetermined. However, the patient survived. One case developed a paralysis of the left leg due to an injection having been given too low in the buttock, and is still in the hospital.

The actual cause of death was unknown in the eight cases which failed to survive. We are in agreement with Adriani and Kerr (1955) that, irrespective of the therapy and care given to patients with tetanus, a certain number of them will die. Three of these cases had no evidence of bronchopneumonia and were classified as dying of toxemia and exhaustion. The other five had a complicating bronchopneumonia.

Adriani and Kerr (1955) have pointed out that phenobarbital is not the drug of choice in the management of tetanus and we are in agreement but continued to use it due to a lack of trained personnel. Tubocurare (Adriani and Ochsner, 1947) was used for 2 days in one case. However, we found it too dangerous in unskilled hands.

We have had little experience with the combination of mephenesin and chlorpromazine (Adriani and Kerr, 1955) and have only recently used it on two cases. Calcium gluconate was used in 20 of our cases, being given intravenously since the laboratory was unable to do Ca and P determinations to rule out tetany. It proved to be of no value. Fourteen cases received 12.5 mg of cortisone intramuscularly daily for a period of 2 weeks. We are unable to evaluate its effects in lessening the rigidity. In none of the cases was a tracheotomy performed. Laryngeal spasm in neonatal tetanus does not seem to be as severe as that found in older children with tetanus. After 6 to 8 days chlorpromazine was used orally and given in the same dosage, one mg per kg every 4 hours as necessary until the muscular rigidity had disappeared entirely. Sodium phenobarbital, 16 to 32 mg, was given intramuscularly every 6 hours as necessary for an average of 8 days, varying from 3 to 15 days.

SUMMARY

The management and complications of 32 cases of tetanus neonatorum with eight deaths are discussed. Tetanus is a preventable disease and in countries where doctors are not available for deliveries every effort should be made to train midwives in the proper care of the newborn.

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STUDIES ON *PASTEURELLA PESTIS* IN FLEAS VII. THE PLAGUE-VECTOR EFFICIENCY OF *HYSTRICHOPSYLLA LINSDALEI* COMPARED WITH *XENOPSYLLA CHEOPIS* UNDER EXPERIMENTAL CONDITIONS[1]

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Ecological studies of wild-rodent plague in the San Francisco Bay region have shown that the flea, *Hystrichopsylla linsdalei*, is consistently infected with plague during epizootics among small native voles and field mice, and that it may be an important vector in the maintenance of enzootic plague in this area (Miles et al., 1957; Kartman et al., 1958a; Kartman et al., 1958b). An essential aspect of these ecological studies is the experimental determination of the vector capacity of wild-rodent fleas which can be correlated with field studies. Laboratory tests with a number of fleas have already been reported, including experiments with *H. linsdalei* (Kartman et al., 1956; Kartman and Prince, 1956). Since *H. linsdalei* is thought to be a primary vector of wild-rodent plague in this area, further tests of its vector capacity were undertaken. The basis for comparison in these experiments, as in the past, has been the so-called classical plague vector, *Xenopsylla cheopis*.

MATERIALS AND METHODS

The detailed methods and materials used in these studies have been described in previous reports (Link and Prince, 1950; Kartman et al., 1956; Kartman and Prince, 1956).

The wild-rodent flea, *H. linsdalei*, was obtained as adults from the nests of the native vole, *Microtus californicus*, in the San Francisco Bay region. It is reasonable to conclude that none of the individual fleas used was already infected with plague in the field since each flea was observed under a microscope after its initial feeding on infected laboratory mice. If plague-like masses had been present they could have been readily seen at that time. This was made even more certain since the fleas were held from 7 to 15 days in the laboratory before being used.

The *X. cheopis* were from the stock colony maintained in this laboratory and were originally obtained from domestic rats in San Francisco.

The infected fleas were maintained individually in test tubes in a room where hygrothermographic records throughout the experiment showed an average temperature fluctuation between 18°C and 21°C with a total average of 19°C. The relative humidity averaged 55 per cent.

The plague organism used in these tests was *Pasteurella pestis* strain BAS 744, a virulent strain originally isolated from fleas collected in the San Francisco Bay area and maintained in this laboratory. Approximately 2-month-old albino mice were inoculated intraperitoneally with 0.5 ml of a 10^{-3} dilution of the *P. pestis* culture and in 36 to 48 hours most of the mice exhibited terminal septicemia when tail blood smears revealed from 5 to 10 or more bacilli per microscopic field. At this time the fleas were fed on some of the moribund mice in batches of about 25 to 40 fleas per mouse. Mouse numbers 1, 2, 5, and 9 were used to feed the *H. linsdalei*, whereas mouse numbers 7, and 9 were used to feed the *X. cheopis*. Fleas of both species were placed simultaneously on mouse No. 9. Mouse No. 5 died within 10 minutes after *H. linsdalei* had been placed on it. Fleas of both species were removed from mouse No. 9 after 25 minutes and the mouse was killed. All other mice died within 30 minutes after fleas had been placed on them for the infectious feeding. Septicemic infection in all the mice used was confirmed by bacteriological culture.

A sample consisting of six *H. linsdalei* and four *X. cheopis* was taken within from 2 to 4 hours after the infectious feeding and the individual fleas were triturated and cultured on blood agar. All of these fleas showed the presence of heavy infections with *P. pestis*. As might be expected, since *H. linsdalei* is a much larger flea than *X. cheopis*, the former ingested more blood and more plague organisms. In a previous report (Quan et al., 1954) it was shown that the average weight per flea of 50 male *X. cheopis* was 0.30 mg,

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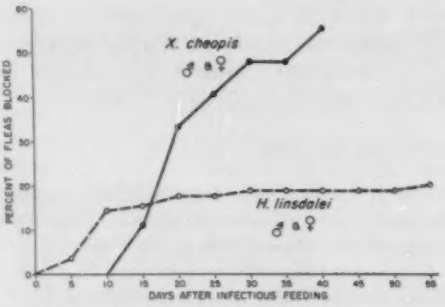

FIG. 1. The cumulative blocking rate of fleas, *Hystrichopsylla linsdalei* and *Xenopsylla cheopis*, at various days after an infectious feeding on mice with terminal plague septicemia.

TABLE 1

The experimental plague-vector index of fleas, Hystrichopsylla linsdalei *and* Xenopsylla cheopis, *when calculated by the relation:*

$$\frac{\text{Number of transmissions}}{\text{Number of fleas infected}}$$

Flea species	Sex	Number infected	Number transmissions	Vector index
H. linsdalei	♂	43	0	0
	♀	41	3	0.07
	♂ + ♀	84	3	0.03
X. cheopis	♂	17	2	0.11
	♀	10	7	0.70
	♂ + ♀	27	9	0.33

whereas 50 females averaged 0.27 mg. Nevertheless, in spite of a lesser body density the females had a greater stomach capacity. In contrast to this, 25 unfed male *H. linsdalei* showed an average weight per flea of 1.36 mg, and 25 females averaged 2.44 mg. The females ingested 1.10 mg blood per flea, whereas the males ingested 0.59 mg blood per flea.

As in previous work, individual fleas were fed upon single mice throughout the course of the experiment. The mice were held for at least 10 days and, if they had not succumbed to confirmed plague infection, were then killed and tested bacteriologically for the presence of plague organisms. *P. pestis* infection in each flea was determined by plate culture either at its death or upon its sacrifice if it survived to the termination of the experiment.

The various criteria for determination of plague vector potentials have already been presented in detail by Kartman et al., (1956) and by Kartman (1957).

DATA AND DISCUSSION

The combined, proventricular blocking rates of male and female fleas of both species are shown in Figure 1. Although the *H. linsdalei* blocked sooner than the *X. cheopis*, the rate of blocking in the latter species was much more rapid and greater numbers of individuals blocked. Actually, by 30 days, the *H. linsdalei* were almost finished with blocking since only one more individual blocked at 55 days; however, this represented only 19 per cent of the infected individuals. On the other hand, by 30 days over 48 per cent of the infected *X. cheopis* had blocked, and 2 additional individuals blocked on the 40th day. Of nine male *H. linsdalei* which blocked, eight showed this condition between the 5th and 20th day, whereas of eight females blocking, seven showed this condition between the 10th and 20th day. Of seven male *X. cheopis* blocking, all had done so between the 15th and 25th day, whereas of the eight females blocking, all showed this condition between days 15 and 40. These results are similar to those in former studies where the male fleas showed more rapid blocking than the females (Kartman et al., 1956).

From the standpoint of plague ecology, the determination of the plague-vector potential of a particular flea species is a complex problem. Some of the implications of this problem have been discussed by Kartman (1957). In the present report, no attempt is made to correlate experimental and field observations.

In the determination of a vector index, a practical method is to relate the number of plague transmissions to the number of infected fleas. This has been done in Table 1 where the indices show the superiority of *X. cheopis* as a plague vector. Some of the reasons for the differences in the vector capacity of the two species are shown in Table 2 where a detailed analysis of the vector indices is given (see Kartman et al., 1956, and Kartman and Prince, 1956). These data show that, in spite of a higher infection potential, the blocking potential of *H. linsdalei* was inferior to that of *X. cheopis*. On the other hand, *H. linsdalei* had a shorter incubation period (14 days) than *X. cheopis* (21 days) and, in terms of actual blocked individuals available, there were 17 for

TABLE 2

Analysis of the experimental plague-vector indices of fleas, Hystrichopsylla linsdalei *and* Xenopsylla cheopis, *when fed on albino laboratory mice and held at an average temperature of 19°C*

Flea species	Sex	No. used	No. infect.	No. block.	No. trans.	Blocking/ survival ratio*	Infect. poten.	Block. poten.	Trans. poten.	Block./ survival poten.	Vector index†
H. linsdalei	♂	47	43	9	0	1.22/13.88	0.91	0.20	0	0.09	0
	♀	61	41	8	3	1.25/15.50	0.67	0.19	0.37	0.08	0.004
	♂ + ♀	108	84	17	3	1.23/14.64	0.77	0.20	0.17	0.08	0.002
X. cheopis	♂	31	17	7	2	2.85/18.12	0.54	0.41	0.28	0.16	0.01
	♀	19	10	8	7	3.75/25.12	0.52	0.80	0.87	0.15	0.05
	♂ + ♀	50	27	15	9	3.33/21.86	0.54	0.55	0.60	0.15	0.03

* Mean day of death after blocking/mean day of blocking after infectious meal.
† Product of the four potentials.

the former and 15 for the latter species. Thus the question arises, why were there fewer plague transmissions by *H. linsdalei*? A partial answer, at least, may be found in the survival of the blocked fleas. Table 2 shows that blocked *H. linsdalei* had a survival of slightly over 1 day, whereas blocked *X. cheopis* survived over 3 days. This means that the latter species had more opportunities to transmit plague. That this is actually the case is shown in Table 3 which demonstrates that fewer blocked *X. cheopis* had almost two times as many opportunities to transmit than did the blocked *H. linsdalei*. That blocked individuals of *H. linsdalei* are in themselves not particularly inefficient vectors is shown by the transmission-success ratio (Table 3) of the females when compared with the female *X. cheopis*.

It is of interest to note the results obtained in a former test with these species when the temperature averaged 21°C (Kartman and Prince, 1956). In that case *H. linsdalei* showed a vector index of 0.02, and *X. cheopis* an index of 0.06. The fact that *H. linsdalei*, in the two experiments, showed a greater difference than the *X. cheopis* may not be due so much to temperature differences as to the fact that the former species was obtained from the field, since culture methods still have not been worked out. Thus, the age of the fleas and other factors which may have influenced their vector capacity could not be controlled.

It has already been mentioned that the fleas received their original infectious feedings from several mice with terminal plague septicemia. Although all of these mice appeared to have comparable numbers of plague organisms in their

TABLE 3

The plague-transmission success of fleas, Hystrichopsylla linsdalei *and* Xenopsylla cheopis, *in terms of opportunities by blocked individuals to feed on albino mice*

Flea species	Sex	No. blocked	Opportunities to transmit	No. of trans.	Trans. success ratio*
H. linsdalei	♂	9	12	0	0
	♀	8	10	3	0.30
	♂ + ♀	17	22	3	0.13
X. cheopis	♂	7	17	2	0.11
	♀	8	24	7	0.29
	♂ + ♀	15	41	9	0.22

* $\frac{\text{Number of transmissions}}{\text{Opportunities to transmit}}$

TABLE 4

Infection and proventricular blockage in male and female fleas, Hystrichopsylla linsdalei *and* Xenopsylla cheopis, *which had been fed on different albino mice with terminal plague bacteremia**

Type of observation	*H. linsdalei* fed on mouse No.				*X. cheopis* fed on mouse No.	
	1	2	5	9	7	9
No. fleas fed	14	37	23	34	16	34
No. infected	13	33	13	25	6	21
Per cent infected	92.8	89.1	56.5	73.5	37.4	61.8
No. blocked	0	11	0	6	3	12
Per cent blocked	0	33.3	0	24.0	50.0	56.9

* All mice showed about 5 to 10 or more *P. pestis* per microscopic field on slides from tail blood; plate cultures of a loopful of tail blood resulted in confluent growth of *P. pestis*.

blood when the fleas were fed, the question was raised whether blocking had been influenced by the mice through possible differences in the infection derived by the fleas. Table 4 summarizes the percentage infection and blocking of *H. linsdalei* and *X. cheopis* after feeding on different mice. These data show that, in almost all cases, the *H. linsdalei* had higher infection rates than the *X. cheopis* regardless of the mouse on which they had fed. This is particularly evident in the case of mouse No. 9 upon which both flea species were fed simultaneously. In this instance exactly the same number of individuals of each species took a blood meal; thus the infection and blocking rates were affected by a minimum of variables. Nevertheless, in this and in all cases where blocking occurred, it was lower in the *H. linsdalei* than in the *X. cheopis*. It might be tempting to ascribe this difference purely to chance since the numbers of *H. linsdalei* which fed on animals No. 1 and No. 5 were small. On the other hand, the number of *X. cheopis* feeding on mouse No. 7 was also small, yet 50 per cent of these fleas blocked. Thus, the existing evidence points to fundamental physiological differences in these two flea species which are at present not known.

Unpublished work at this laboratory has suggested that a threshold may exist in the number of plague organisms, ingested by a flea, which determines subsequent blocking. Thus, mere infection and intensity of infection in an individual flea may be distinct, though related, factors. It is possible that the threshold for *H. linsdalei* is quite different from that for *X. cheopis* and that this may account for the fact that high infection rates in the former species did not necessarily lead to high blocking rates.

Of three mouse deaths following plague-infective feedings by *H. linsdalei*, the times of death were 4, 5, and 6 days, respectively, with a mean life of 5 days after infection. Of nine mouse deaths following infective feedings by *X. cheopis*, the times of death were 3, 3, 3, 3, 4, 5, 5, 5, and 7 days, respectively, with a mean life of 4.2 days after infection. The figures for *X. cheopis* are virtually the same as those obtained in a previous test (Kartman et al., 1956) in which the mean day of death was 4 days. At that time it was shown that there was a marked likeness between the time and percentage mortality curves of mice dying of plague from flea infection and those dying after intracutaneous inoculation of 0.05 ml of an aqueous suspension containing about 3×10^3 virulent *P. pestis*. Since, in the case of *H. linsdalei*, very few plague transmissions and mouse deaths occurred, it would be difficult to evaluate this problem. Additional data (unpublished) bearing on this point may be cited from former experiments with this flea species (Kartman and Prince, 1956). Of six mouse deaths following plague-infective feedings, the times of death were 4, 3, 3, 7, 8, and 4, respectively, with a mean life of 4.8 days after infection. Thus, the average life of the mice is closer to the present figure for *H. linsdalei* than to that for *X. cheopis*. If the mouse-death times in the present experi-

TABLE 5

Results of fecal cultures from fleas, Hystrichopsylla linsdalei, *alive 10 or more days after initial feeding on plague-infected white mice*

Flea No.	No. times feces were		Flea died on day	Flea culture was	Visual criteria*
	Negative	Positive			
	Males				
29	7	1	17	Neg.	o
32	1	5	16	Pos.	m
35	2	13	56	Pos.	b
36	0	6	15	Pos.	m
46	0	9	21	Pos.	b
49	0	2	14	Pos.	b
50	20	0	50	Neg.	o
51	6	0	16	Neg.	o
55	0	8	16	Pos.	m
	Females				
3	0	6	16	Pos.	m
5	0	11	71	Pos.	m
6	14	0	48	Neg.	o
9	14	1	61	Neg.	m
10	0	4	15	Pos.	m
11	1	8	30	Pos.	b
13	15	0	48	Neg.	m
27	11	1	67	Neg.	m
33	2	6	20	Pos.	m
36	0	3	12	Pos.	m
39	1	8	21	Pos.	b

* Microscopic observations showed these fleas to be blocked (b), to contain apparent plague masses (m), or never to have developed any signs of plague growth in their intestinal tract (o).

ment are combined with those in the former, the mean life is approximately the same, 4.9 days. Thus, there is a suggestion that blocked *H. linsdalei* may, on the average, inject fewer plague organisms than *X. cheopis*. If this is true, it would constitute yet another biological factor in the vector potential peculiar to a given flea species.

Although culture of the individual flea was used for critical determination of infection with *P. pestis*, culture of flea feces was made at various times; mainly with the *H. linsdalei* since culture of *X. cheopis* feces has been a routine for many years in this laboratory. In general, the culture of feces has not been found to be a reliable means of determining infection since results are variable during the life of the flea (Kartman et al., 1956). Nevertheless, it is of interest to record the relation of fecal culture to culture of the flea and, in addition, to correlate these data with the results of microscopic observations regarding the development of plague masses in the same individual. Representative data of this type are shown in Table 5.

These data suggest the general unreliability of fecal culture as an absolute criterion of infection since, in several cases, both positive and negative results were obtained. A judgment would have to be based on whether negative or positive results predominated, but this would necessarily require many observations over long periods. It is also seen that visual criteria do not always hold true since, in several cases, masses were observed in fleas which proved negative for *P. pestis*. It is of interest that female *H. linsdalei* No. 9 showed masses, was negative for plague, but on culture yielded a pure strain of bacteria unrelated to *P. pestis*. Thus, although fecal culture and microscopic observation are useful procedures, they do not provide an unequivocal basis for the determination of flea infection with *P. pestis*.

SUMMARY

An experimental study is reported on the plague-vector capacity of the wild-rodent flea, *Hystrichopsylla linsdalei*, compared with the rat flea, *Xenopsylla cheopis*. The average temperature under which the infected fleas were held (19°C) was lower than that in previous tests with *H. linsdalei*.

Although the *H. linsdalei* blocked sooner than the *X. cheopis*, the rate of blocking in the latter species was more rapid and resulted in a greater number of blocked individuals.

The plague-vector indices of male and female fleas combined were 0.002 for *H. linsdalei* and 0.03 for *X. cheopis*. The lower efficiency of *H. linsdalei*, under the conditions of the experiment, are partially ascribed to a lower blocking rate and a shorter survival period for the blocked individuals. Thus, the wild rodent flea had fewer opportunities to transmit *P. pestis* than the rat flea.

There was no evidence that infectious feedings by the fleas on different mice with comparable plague septicemia influenced subsequent infection or blocking rates. Rather, differences between the two species suggested the presence of unknown physiological factors which may enter into the receptivity of a flea species to the growth of plague within its intestinal tract.

The average death time of mice succumbing to plague transmitted by *H. linsdalei* was about 5 days, whereas the death time for mice infected by *X. cheopis* averaged about 4 days. This may have some bearing on the number of bacilli injected by blocked fleas.

In confirmation of past work, it was found that culture of feces from, and microscopic observation of, fleas infected with *P. pestis* are useful procedures, but they do not provide an unequivocal basis for the determination of individual flea infection. The culture of individual fleas is the method of choice.

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SEROLOGICAL SURVEY FOR ARTHROPOD-BORNE VIRUS INFECTIONS IN THE PHILIPPINES[1]

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Virology in the Philippines in recent years has dealt almost entirely with rabies and to a lesser extent with influenza except for a short period following World War II when a U. S. Army Medical laboratory in Manila operated a virology section. During this period there was a reported isolation of Eastern equine encephalomyelitis (EEE) virus from a pet monkey in Manila (Livesay, 1949). Some serological studies on virus infections were also carried out in this laboratory (Mace et al., 1949). Subsequently, diagnostic specimens, chiefly serological from Americans in the Philippines, were handled at the Army's 406th Medical General Laboratory in Japan. Several cases of encephalitis were diagnosed serologically in this laboratory as due to Japanese B encephalitis (JBE) virus. The virus, however, was never isolated. Previously, JBE antibodies in Philippine horses were reported by the Japanese (Mitamura, 1943), and Hammon (1954) found a high prevalence of JBE-neutralizing antibodies among Philippine Scouts newly arrived on Okinawa in 1947 and in Filipinos in Manila and California. A much more complete review of viral infections of the central nervous system was published previously (Hammon, 1954).

Many years ago outstanding experimental work with dengue virus, then endemic in the Manila area, was carried out by U. S. Army medical and laboratory personnel, using human volunteers, mosquitoes and monkeys (Siler et al., 1926; Simmons et al., 1931). No further work was done with dengue virus in this area, however, after it was adapted to mice and in vitro tests became available (Sabin and Schlesinger, 1945; Sabin, 1950). It was the consensus of local authorities in 1956 that the virus was no longer present and had been absent for many years, except for its probable reintroduction during World War II and its prompt disappearance thereafter.

During a research expedition in 1953 planned to obtain information on the epidemiology of poliomyelitis at Clark Air Force Base (CAFB), located about 60 miles north of Manila, sera were collected from several hundred Filipino and Negrito natives. These were purposely obtained from three quite different ecological areas in respect to probable mosquito breeding and prevalence. These sera have been tested, in so far as possible, against the three types of poliovirus (Hammon, W. McD., Schrack, W. D., Jr., and Ludwig, E. H., unpublished data) and 15 selected arthropod-borne viruses in an attempt to determine the groups or types of viruses active, and some general concepts as to their prevalence and distribution. Tests against the 15 arthropod-borne viruses are reported here.

POPULATION SAMPLE

The three population groups bled were: (1) practically all inhabitants of two Negrito villages, the one located in jungle and mountain foothills just north of CAFB, and another just across the southwest perimeter fence of the base; (2) a group of Filipino school children aged 10–14 years (originally 9–15 but, for purposes of analysis, all 9 and 15-year-olds were eliminated) from the village of Sapangbato, immediately adjacent to CAFB (along the south perimeter fence) in an area of open fields and, (3) Filipino children aged 6 months to 14 years from Manila, attending clinics at the Philippine General Hospital, not including any suspected of having poliomyelitis or encephalitis. A fourth group, not so well defined ecologically and therefore of less value, was composed of normal food handlers aged 15 and above; a

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few from Manila but mostly from CAFB. Histories were obtained from all these to determine that they had always been residents of the Philippines and had not traveled elsewhere. Since many of these resided in Sapangbato and most were from that general area they will be considered as population of that village, and results as presented will be included with those of Sapangbato.

The Negrito population was primitive in customs and habits and the natives of all ages roved almost continuously during the day in jungle and foothill mountain areas hunting, working gardens or visiting. One Negrito village was adjacent to the military base. Sapangbato was a very dilapidated and squalid village by Filipino standards, constantly "off limits" to the military. The adults and older children were often seen in gardens and rural areas at a distance from the village, but many remained most of the time in or near the village. They did not have the jungle exposure of the Negritos. The village, because of its proximity to inhabited portions of the military base, benefited from a measure of military mosquito control.

Manila at this time was still attempting to rebuild after the extensive war damage which had hit certain sections severely. Hundreds of thousands of rural residents had swarmed into Manila for protection from "Huk" raiders. Living conditions varied from the most modern for a few to essentially no roof for many more. Thousands lived in holes or caves in destroyed walls and in shanties constructed of old packing cases or sheets of discarded metal. Malaria did not occur at this elevation and no other mosquito-borne disease was recognized as a problem, so mosquito control was nonexistent.

METHODS

All sera were frozen at about −20°C after separating from the cells and were maintained at this temperature, or at that of a dry ice box, until tested at Pittsburgh.

The neutralization test was selected for principal use despite its much greater expense and labor. It was known to be the most specific and, under circumstances such as these, no advantage could be seen in screening by less specific means. However, since dengue was less closely related to JBE virus than many others of the group B agents as classified by Casals and Brown (1954), the hemagglutination inhibition (HAI) test of Sweet and Sabin (1954) was employed first for dengue, then subsequently confirmed with a generous sample using the suckling-mouse neutralization test.

In all neutralization tests two dilutions of virus were first employed as a screen test. Many of those which had a neutralization index greater than 50 were titrated by the virus-dilution method to an endpoint or at least to an index of 10,000. A neutralization index of 50 or more was considered positive. An index between 25 and 50 was an indication for a repeat test for final classification as positive or negative. The intracerebral test using undiluted, unheated serum incubated at 37° C for 2 hours with the virus, as described previously (Hammon, 1956) was employed except in dengue. Here the only change was that suckling mice 2 or 3 days of age replaced adult mice.

Complement-fixation tests using benzene-extracted antigens (Espana and Hammon, 1948) by a modified drop technic (Hammon, 1956) were performed on many of the sera found positive to one or more viruses by neutralization test. Antigens used were Western equine encephalomyelitis (WEE), JBE, Murray Valley encephalitis (MVE), West Nile (WN), St. Louis encephalitis (SLE), "Epidemic keratoconjunctivitis" of Saunders (EK) and dengue type 1.

RESULTS

Group B

Table 1 and Figure 1 show the neutralization-test findings for group B viruses in the three areas, by age groups and by virus. Uganda S was omitted from the figure since there were no positives found to this virus. It should be noted that among Negritos the 10–14-year-age group is small in number and for this reason the percentage positive as recorded is possibly out of line in several instances. It probably, therefore, should be judged to fall between that of the 5–9 and the 15–19-year age groups which, because of the larger samples, are much more stable. Numbers in all age groups in other areas are large enough to minimize sampling error.

JBE—Among the Negritos, antibodies to JBE occur most frequently and in highest titer; therefore, we may assume that one of the

TABLE 1

Group B viruses. Positive results of neutralization tests

Locality	Age (yrs)	JBE		Dengue*		MVE		WN		Ntaya		Zika		SLE		Ilheus		Uganda S	
		Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
Negritos Jungle	6/12–4	7/18	39	4/14	29	1/14	7	2/12	17	1/11	9	0/11	0	0/10	0	1/10	10	0/4	0
	5–9	18/23	78	7/19	37	2/19	10	2/15	13	4/20	20	0/19	0	0/10	0	0/20	0	0/17	0
	10–14	5/6	83	4/6	67	3/6	50	2/4	50	2/6	33	1/6	17	1/6	17	1/6	17	0/4	0
	15–19	15/15	100	6/12	50	7/15	47	6/15	40	4/11	36	3/11	27	3/15	20	2/11	18	0/3	0
	20+	61/62	98	38/54	70	34/62	55	36/62	58	28/53	53	8/51	16	21/61	34	11/53	19	0/13	0
Sapang-bato Fields	10–14	12/52	23	22/40	55	8/52	15	6/52	11	8/29	28	3/24	12	5/31	16	0/26	0	0/16	0
	15+†	25/51	49	—		—		—		6/12	50	3/12	25	—		1/12	8	0/12	0
Manila Urban	6/12–4	1/32	3	12/33	36	1/26	4	3/27	11	5/22	23	1/13	8	1/23	4	0/18	0	0/2	0
	5–9	4/23	17	13/21	62	4/21	19	6/21	29	7/13	54	0/6	0	5/20	25	0/7	0	—	0
Totals		148/282	52	106/199	53	60/215	28	63/208	30	65/177	37	19/153	12	36/176	20	16/163	10	0/71	0

* Combined results of HAI and neutralization tests with viruses of both types.

† Food handlers principally from this general area.

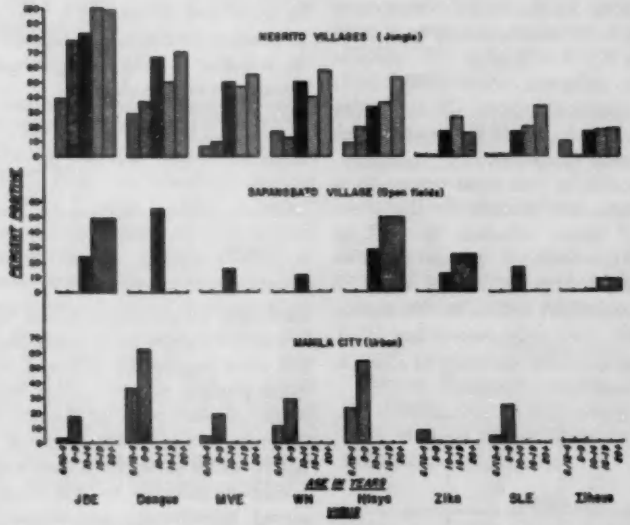

FIG. 1. Results of neutralization tests with group B viruses by area and by age group.

viruses present is most closely related to JBE. Since it has been isolated from Malaya, Guam and the Ryukyus, it can be regarded as probably present here on the basis of this serological evidence.

Dengue—This virus has been recognized in years past as the cause of many epidemics among foreigners. It accounted for epidemics among Japanese during World War II occupation and then among Americans after the departure of the Japanese. Since World War II it has not been observed. Physicians in Manila state that this disease does not occur among Filipinos. Antibodies shown in the figure are

the combined results of neutralization tests and HAI tests with each of the two viruses. There was essentially complete agreement between the two kinds of tests and only a few individuals had antibody to one type of dengue virus (type 1 or 2) without an almost equal titer to the other.

It will be observed that the dengue antibodies found in Sapangbato and in Manila cannot be interpreted as a reflection of the presence of JBE antibody for the prevalence of the dengue antibodies is much higher than that of the latter. Furthermore, evidence of this independence is manifest by noting that the prevalence of dengue antibodies in Sapangbato and in Manila is probably higher than that present in Negritos (approximately 45% appears to be a reasonable estimate for Negritos aged 10–14 years), while JBE antibodies are present much more frequently in Negritos. Many individuals without JBE antibody were observed to possess dengue antibodies and, among those with both, the titer to either may be the higher. Of 96 sera tested for dengue CF antibodies only 4 were positive in titers of 1:8 or greater. The interpretation of this is unknown. Sabin (1956; 1957, personal communication) reports CF antibodies remaining for at least 4 years in the experimental disease with human volunteers.

MVE—Antibodies to this virus appear in a relatively proportionate manner in the three areas and could reflect infection by JBE or another agent. However, 61 persons positive to JBE were negative to MVE and seven of these JBE positives had neutralization indices in excess of 10,000. Also, eight persons had MVE antibody who did not have antibody to JBE. A fourfold association table is shown.

		JBE	
		0	+
MVE	0	97	61
	+	8	55

WN—Antibodies to WN behave much like those of MVE. Again, 62 persons with JBE antibody did not possess any for WN even though eight had titers above 10,000. Eleven possessed WN antibody without JBE. An association table is shown.

		JBE	
		0	+
WN	0	92	62
	+	11	52

The same virus could well have accounted for MVE and WN antibody, but this probably was not JBE virus.

Ntaya—Antibody to this virus certainly does not stem principally from JBE infection. It is most prevalent in Manila, ranking second only to dengue. In Sapangbato it also ranks second (to dengue) while its prevalence is fourth or fifth in order among Negritos. There appears to be a strong probability that these antibodies are caused, in part at least, by a virus not associated with other antibodies since 12 persons had antibody to Ntaya and none to others of group B. Some of these were not tested for dengue, however. That these antibodies cannot be attributed to dengue is also apparent since 16 positive for Ntaya are negative to dengue and 41 negative to Ntaya are dengue-positive. An association table is shown.

		Dengue	
		0	+
Ntaya	0	53	41
	+	16	45

Obviously no association occurs here.

Zika—Very few have antibodies to this virus, but three persons did who had no antibodies to other group-B agents against which tests were made. Absence from 30 Negrito children under 10 years of age tested suggested that it might not be active currently in their area.

SLE—Antibodies to this virus also are observed infrequently, are absent from Negrito children under 10 years of age and appear to be most prevalent in Manila. They occur in the absence of JBE but not of both JBE and dengue.

Ilheus—Antibodies to this virus were found only among Negritos and a few adult food handlers. Only twice did they occur without JBE and in both instances dengue antibody was present. Like Zika and SLE there appears

TABLE 2

Group A and miscellaneous viruses. Positive results of neutralization tests

Locality	Age (yrs)	Group A						Miscellaneous			
		WEE		EEE		Semliki Forest		Bunyamwera		Bwamba	
		Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
Negritos	6/12–4	—		0/5	0	2/6	33	0/4	0	0/4	0
	5–9	—		0/5	0	4/18	22	0/17	0	0/17	0
	10–14	—		0/4	0	0/6	0	0/4	0	0/4	0
	15–19	—		0/11	0	2/9	22	0/3	0	0/3	0
	20+	—		0/26	0	8/41	20	0/9	0	0/13	0
Sapangbato	10–14	0/18	0	0/46	0	0/17	0	0/13	0	0/8	0
	15+*	—		0/34	0	2/12	17	0/12	0	0/12	0
Manila	6/12–4	—		0/30	0	0/8	0	0/2	0	0/2	0
	5–9	—		0/24	0	0/2	0	—		—	
Total		0/18	0	0/185	0	18/119	15	0/64	0	0/63	0

* Food handlers principally from this general area.

to be little evidence of its presence in children, therefore it might result from a combination of group-B infections.

Group A

Only three members of group A were employed and the results are shown in Table 2.

EEE—Antibodies for this virus were anticipated since EEE virus was reportedly isolated from a monkey in Manila in 1947 (Livesay, 1949). However, despite tests on 185 persons in these areas during the present studies, no antibodies were found. Since antibodies for EEE were previously reported for horses on Luzon (Mace et al., 1949), blood samples were collected from 24 horses and 18 carabao near Clark Air Force Base in 1956. The results of tests with these are shown in Table 3. Finding antibodies adds further support to the reported isolation of the virus. Apparently man is seldom bitten by the vector of this virus, as in the United States.

WEE—Eighteen sera from Sapangbato were negative when tested against WEE. Serum from one of the 24 horses bled in 1956 had a neutralization index of 100. The 18 carabao were negative.

Semliki Forest—Antibodies for this group-A virus were found only among the Negritos and in adult food handlers. However, the numbers positive to this one group-A virus probably have some real significance, though the virus responsible may not actually be Semliki Forest virus, but one closely related to it.

TABLE 3

Horses and carabao sera. EEE virus. Positive results of neutralization tests

Species	Ratio	%	Range of positive NI's*
Horse.....	7/24	29	60–2,000
Carabao.....	1/18	6	100

* Neutralization indices.

Miscellaneous

Among the known viruses not yet classified by Casals only Bunyamwera and Bwamba were used. As shown in Table 2 all tested were negative.

SUMMARY

Viruses in this study, because of the many antigenic overlappings, cannot all be identified on the basis of serological response alone. It appears highly probable, however, that JBE, dengue and EEE viruses are present, plus at least two others from group B (one of these possibly Ntaya) and at least one other from group A, closely related to Semliki Forest virus if not that virus. As a result of this preliminary

survey an expedition was organized to attempt to isolate and thus actually identify the agents present. Testing of these latter materials is still under way.

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THERAPY OF TRICHURIASIS AND ASCARIASIS WITH DITHIAZANINE[1]

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Recently McCowen et al., (1957) reported that dithiazanine has anthelmintic activity against ascarids and whipworms of dogs. Frye et al., (1957) obtained high cure rates with this new trichuricide in unquantitated, subclinical, *Trichuris trichiura* infections. The present study was undertaken to evaluate the efficacy of dithiazanine: (a) for treatment of individual cases of severe clinical and of subclinical trichuriasis in which the infections were quantitated by egg counts, (b) for mass therapy of trichuriasis and (c) for treatment of *Ascaris lumbricoides* infection.

Concurrently, the anthelmintic spectrum of dithiazanine has been explored. A cure rate of 89 per cent was reported by Swartzwelder et al., (1958), in a series of cases of human strongyloidiasis treated with this drug. The rigid criteria for cure were the absence of diagnostic forms in a series of examinations of both stools and duodenal fluids after therapy. Miller et al., (1958) employed dithiazanine successfully for the treatment of enterobiasis. Thus, evidence of the effective broad spectrum or polyvermicidal activity of dithiazanine against common intestinal helminths of man has been developed.

ANTHELMINTIC

The drug employed in this study was dithiazanine, 3-3'diethylthiadicarbocyanine iodide. It is a blue powder which is not readily absorbed from the intestine. The anthelmintic was administered in almost all cases in 100-mg enteric-coated tablets designed to delay a release of the drug until after passage through the stomach. A few patients received the drug in either 50-mg tablets or in a cherry-flavored suspension which contained 20 mg of the anthelmintic per ml.

TRICHURIASIS

METHODS

Individual therapy. Thirty six children with clinical or subclinical whipworm infection were treated in the wards and clinics of a hospital in Louisiana. All except ten of the children weighed less than 40 pounds. Many of these patients had heavy worm burdens and a history of diarrhea or dysentery of long duration. Initially, the dosage employed was 10 mg of dithiazanine per lb of body weight per day, given in divided doses 2 or 3 times daily. Finally, the following schedule was adopted: 20 mg of dithiazanine per lb, with a maximum of 600 mg per day, given in divided doses 3 times daily (*i.e.*, 200 mg *t.i.d.*), for 5 or more days depending on the intensity and severity of the infection (Table 1).

Mass therapy. A total of 262 persons in five units of an institution for the mentally retarded were treated in groups to determine the effectiveness of this new anthelmintic for mass therapy. *T. trichiura* infection was present in 201 of these patients. All of the persons treated weighed more than 90 lbs. Most of the whipworm infections were subclinical, but a few persons had worm burdens sufficient to produce symptoms. The drug was given for 1, 2, 3, 5 and 10 days at a dosage of 200 mg three times daily (Table 2).

Quantitation and evaluation. When patients were treated individually at the hospital, stools were collected prior to, during and for one or more weeks after completion of therapy. Stoll egg count and formalin-ether techniques were done promptly on the fresh, unpreserved specimens. Adult trichurids passed during therapy were collected. Urinalyses were done before and after treatment.

When the studies on mass therapy for tri-

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The drug employed in this study, dithiazanine, was supplied by Eli Lilly and Co.

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TABLE 1

Trichuriasis treated with dithiazanine

Case No.	Age (yrs)	Wgt. (lbs.)	Dosage (*mg t.i.d. or b.i.d. × days*)	Pretreatment egg count/ml	Post-treatment egg count/ml
1	3	26	100 t.i.d. × 5 & 250 b.i.d. × 5	148,700	900
2	10	44	200 b.i.d. × 6 & 100 t.i.d. × 4	134,500	10,200
3	4	28	200 t.i.d. × 12	123,900	0
4	4	31	100 t.i.d. × 6	84,700	0
5	2	20	200 b.i.d. × 10	50,500	0
6	2	22	200 b.i.d. × 10	47,300	0
7	2	21	50 t.i.d. × 5 & 100 b.i.d. × 12	36,200	0
8	2	24	160 t.i.d. × 9	35,900	2,600
9	4	27	100 t.i.d. × 5 & 200 t.i.d. × 5	28,300	0
10	5	33	200 b.i.d. × 5	25,900	0
11	6	38	200 b.i.d. × 5	20,700	0
12	4	34	200 t.i.d. × 5	18,000	0
13	2	23	50 t.i.d. × 5 200 b.i.d. × 5 & 160 t.i.d. × 6	14,900	0
14	2	22	200 b.i.d. × 5	13,400	600
15	2	29	300 b.i.d. × 5	11,600	0
16	—	31	300 b.i.d. × 5	11,100	0
17	2	25	100 t.i.d. × 5	9,900	0
18	8	45	200 t.i.d. × 5	9,700	0
19	5	40	300 b.i.d. × 5	8,600	0
20	2	25	125 b.i.d. × 5	8,600	1,000
21	5	42	300 b.i.d. × 10	7,900	0
22	2	26	100 t.i.d. × 5	6,500	300
23	3	24	200 b.i.d. × 5	5,300	0
24	6	36	300 b.i.d. × 5	4,400	0
25	2	27	200 b.i.d. × 5	3,800	0
26	6	37	300 b.i.d. × 5	2,900	0
27	2	27	250 b.i.d. × 5	1,800	100
28	10	61	300 b.i.d. × 10	1,700	100
29	8	55	300 b.i.d. × 3	1,400	0
30	2	26	250 b.i.d. × 5	1,100	0
31	6	45	300 b.i.d. × 5	1,000	0
32	7	47	300 b.i.d. × 5	800	0
33	5	39	300 b.i.d. × 5	700	0
34	6	50	100 t.i.d. × 2	700	100
35	3	33	200 t.i.d. × 5	600	200
36	8	54	300 b.i.d. × 5	300	0
Total of pretreatment egg counts.....				883,300	
Total of post-treatment egg counts.....				16,100	
Total reduction of egg counts.....				867,200	
Percentage reduction of total egg counts.				98.2	

churiasis were conducted under institutional conditions, the stools were collected before, and 1 to 2 weeks after, treatment. They were preserved in formalin immediately after passage for subsequent examination. The packed sediments obtained by centrifugation were used for formalin-ether concentration and for quantitation of the size of the infections by egg counts. A small number of the patients with trichuriasis in the groups which received mass therapy had light infections which were demonstrable only by formalin-ether concentration; this rendered quantitation of these few infections by egg counts prior to treatment impossible.

RESULTS

Individual therapy. Complete clinical and parasitic cures of trichuriasis were achieved when an adequate dosage of dithiazanine was used. The diarrhea or dysentery terminated. There was a 98.2 per cent reduction of the total egg count in the 36 treated patients. Complete parasitic cure occurred in 26 (72%) cases. Some of the children were released from the hospital before elimination of the entire infection had been obtained. Also, a low dosage was employed during the initial phase of the study. These factors militated against attaining a 100 per cent cure rate in this series. Data on this study are presented in Table 1.

Mass therapy. The cure rates of whipworm infection in groups of persons treated for 1, 2, 3, 5 and 10 days were 3, 24, 77, 97 and 100 per cent, respectively. Only a single *T. trichiura* egg was found in the formalin-ether concentrate of the stool of one patient in the group which was treated for 5 days. The results of therapy are revealed more clearly by analysis of the reduction of total egg count in those cases in which worm burdens were sufficient for quantitation before treatment. The percentages of reduction of total egg count after therapy were 52 (1 day), 88 (2 days), 99 (3 days) and 100 (5 and 10 days). These data indicate that almost all of the whipworms were eliminated by treatment with dithiazanine for 3 days. Significant reduction of the worm burden resulted from therapy for 1 and 2 days. A summary of the study on mass therapy of trichuriasis appears in Table 2.

Ascariasis

METHODS

Thirty of the 36 children with trichuriasis who were treated with dithiazanine in the hospital wards and clinics also had ascariasis. An additional 7 clinic patients parasitized only

by *A. lumbricoides* were given the same drug. Thus, observations of the anthelmintic efficacy of dithiazanine against ascariasis were obtained in 37 patients treated individually (Table 3). The same methods were employed to quantitate the *Ascaris* infections and evaluate the effect of therapy on this helminthiasis in all cases as in the analogous study on trichuriasis.

Fifteen persons in the groups treated for 1, 2 and 3 days during mass therapy for trichuriasis also had ascariasis. Thus, information on the effect of short courses of medication on the latter infection was obtained.

RESULTS

There was a 96.2 per cent reduction in the total *Ascaris* egg count of 37 clinic and ward patients treated with dithiazanine for 5 or 10 days (Table 3). Complete parasitic cure was obtained in 24 (65%) cases. The reduction of

TABLE 2

*Mass therapy of trichuriasis with dithiazanine**

Days of therapy	Total dosage (gms.)	Cases treated	Cases positive after therapy	Total egg count before therapy	Total egg count after therapy	Percentage of egg count reduction†	Percentage of cures
1	0.6	35	34	91,200	43,200	52	3
2	1.2	45	34	161,900	19,900	88	24
3	1.8	52	12	62,200	200	99	77
5	3.0	32	1‡	60,700	0	100	97‡
10	6.0	37	0	76,250	0	100	100

* Dosage: 200 mg t.i.d.

† Based upon all cases with worm burdens sufficient to quantitate by egg counts before treatment.

‡ One egg found in formalin-ether concentrate after treatment.

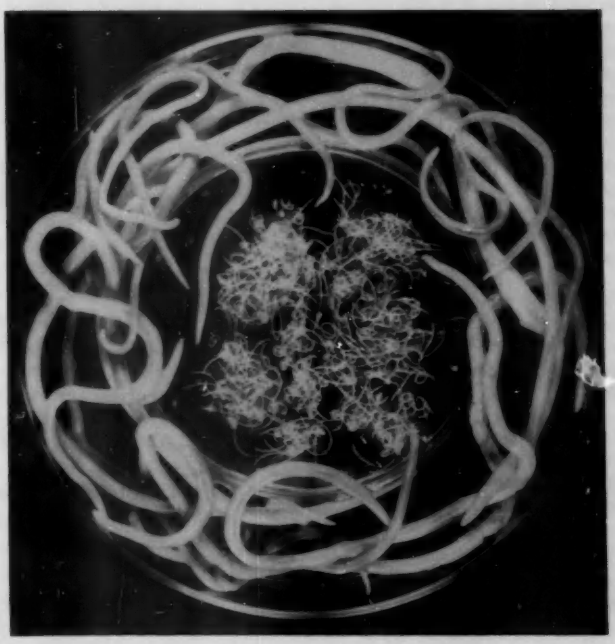

FIG. 1. *Trichuris trichiura* (whipworm) and *Ascaris lumbricoides* adults passed by a child during treatment with dithiazanine.

TABLE 3

Ascariasis treated with dithiazanine

Case no.	Age (yrs)	Wgt. (lbs.)	Dosage (*mg t.i.d. or b.i.d. × days*)	Pretreatment egg count/ml	Post-treatment egg count/ml
1	3	33	200 t.i.d. × 5	199,400	2,700
2	4	28	200 t.i.d. × 10	170,100	0
3	5	40	300 b.i.d. × 10	160,700	5,500
4	5	40	300 b.i.d. × 10	106,100	0
5	5	42	300 b.i.d. × 5	99,100	0
6	2	27	200 b.i.d. × 5	89,800	0
7	2	20	200 b.i.d. × 10	81,200	900
8	2	27	250 b.i.d. × 5	79,500	6,100
9	2	25	200 b.i.d. × 5	72,400	0
10	2	25	125 b.i.d. × 5	60,100	2,400
11	6	37	300 b.i.d. × 5	59,800	0
12	8	54	300 b.i.d. × 5	59,500	6,200
13	6	45	300 b.i.d. × 5	54,200	0
14	5	33	200 b.i.d. × 5	49,800	0
15	10	61	300 b.i.d. × 10	47,500	5,500
16	2	26	250 b.i.d. × 5	39,700	0
17	2	25	125 b.i.d. × 5	35,900	15,600
18	7	48	300 b.i.d. × 5	30,500	7,100
19	2	21	50 t.i.d. × 5 & 100 b.i.d. × 12	26,000	0
20	3	26	100 t.i.d. × 5	24,100	0
21	2	22	200 b.i.d. × 5	20,800	0
22	11	62	200 t.i.d. × 5	18,400	0
23	2	25	100 t.i.d. × 5	14,300	0
24	6	38	200 b.i.d. × 5	13,500	1,000
25	3	30	300 b.i.d. × 5	13,100	0
26	—	31	300 b.i.d. × 5	12,800	0
27	4	34	200 t.i.d. × 10	12,100	0
28	2	27	100 t.i.d. × 5	11,900	2,900
29	2	22	200 b.i.d. × 5	10,800	5,900
30	2	24	250 b.i.d. × 5	8,600	0
31	2	26	100 t.i.d. × 5	5,800	0
32	6	40	100 t.i.d. × 5 & 200 t.i.d. × 5	4,100	0
33	2	29	300 t.i.d. × 5	3,200	0
34	10	44	200 t.i.d. × 6	1,900	0
35	2	23	50 t.i.d. × 5	1,100	0
36	4	27	100 t.i.d. × 5	1,000	1,000
37	8	45	200 t.i.d. × 5	700	0

Total of pretreatment egg counts	1,699,500
Total of post-treatment egg counts	64,600
Total reduction of egg counts	1,634,900
Percentage reduction of egg counts	96.2

the *Ascaris* infections in these patients is significant in view of the fact that the treatment was not directed primarily against ascariasis but was instituted for *Trichuris* infections in all except seven cases. Also, a low dosage of the drug was employed at the beginning of the study. Since most of the children received only 5 days of treatment, a longer course of therapy may be necessary to achieve elimination of all ascarids in some cases. The therapeutic results obtained with dithiazanine (Tables 1, 2, and 3) illustrate clearly that human ascarids and trichurids are amenable to the action of this anthelmintic and that effective therapeutic results may be obtained against mixed infections with both helminths (Fig. 1).

The coincidental observations on the effect of treatment for 1, 2 or 3 days with dithiazanine in 15 cases of ascariasis during mass therapy for whipworm infection are presented in the following summation. There was complete elimination of the ascariasis in one of five cases *treated for 1 day*; the reduction in total egg count in the five cases was 75 per cent. Complete parasitic cure of ascariasis was obtained in seven of eight cases *treated for 2 days* and the percentage of reduction of the egg count in this group was 64. The infection was eliminated in the two remaining persons who were *treated for 3 days*.

TOXICITY AND TOLERANCE

More than 400 patients have been treated by the authors with dithiazanine for 1 to 21 days in these and other studies without the occurrence of any serious untoward reaction. Most of the children and adults tolerated the maximum daily dosage of 200 mg *t.i.d.* for 5 or 10 days without any, or with minimal, side effects. In a closely observed series of small children, many of whom were debilitated by severe trichuriasis, the only adverse reaction was vomiting, which occurred in 9 of 37 patients. However, during the entire study this side effect was observed only occasionally. Usually the vomiting was limited to a single episode during a course of treatment. The reaction was not sufficient to terminate therapy. It is preferable to give the drug about 1 to 2 hours after meals. Diarrhea persisted in three patients with heavy whipworm infection during treatment, but other etiologies such as amebiasis and shigellosis, apparently accounted for this finding. A girl 3 years of age and 40 lbs in weight accidently ingested 14 tablets (1.4 gm) at one time. The child did not become ill or vomit and had no complaints. Routine urinalysis of the

children treated in the hospital wards and clinics for trichuriasis and/or ascariasis showed no discoloration or other abnormality. Since the drug is excreted in the feces, the stools of patients receiving this anthelmintic are colored blue. The drug was removed from the soiled diapers by laundering without residual discoloration.

SUMMARY

Dithiazanine is an effective, oral, therapeutic agent for clinical and subclinical trichuriasis. This new anthelmintic is useful for mass therapy for trichuriasis. Since dithiazanine also has significant anthelmintic activity against *A. lumbricoides*, it is suitable for the treatment of mixed infections of trichuriasis and ascariasis, two helminthiases which frequently coexist in persons in endemic areas. An effective dosage of this drug for the therapy of trichuriasis and/or ascariasis is 20 mg per lb, with a maximum of 600 mg per day given in divided doses 3 times daily (*i.e.*, 200 mg *t.i.d.*), for 5 or more days depending upon the size and severity of the infection. No serious side effects have occurred in several hundred children and adults treated with this anthelmintic.

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THE PREVALENCE OF *TOXOPLASMA* ANTIBODIES IN GUATEMALA AND COSTA RICA

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Although the occurrence of isolated cases of human toxoplasmosis in Latin American countries has been established by a number of investigators, until quite recently there were no reports on the *frequency* with which the infection occurs in those areas. From 1951 to 1953 several Mexican workers, notably Biagi (1951, 1953), Bustos *et al.*, (1952), and Varela *et al.*, (1953) applied the toxoplasmin skin test in several localities of Mexico. They reported the prevalence of positive tests as 15 per cent in Mexico City, 48 per cent at Tampico, 51 per cent at Orizaba, and 57 per cent in the State of Campeche. More recently, Varela *et al.*, (1955) verified these results by use of the Sabin-Feldman (1948) dye test. They found that the prevalence of positive sera in adults varied from 27 per cent in Mexico City, at an elevation of about 7,350 feet, to 65 per cent in Yucatan, where the elevation was less than 1,000 feet above sea level.

In South America, only four studies have been made on the prevalence of *Toxoplasma* in humans. In 1951, Thiermann used the Sabin-Feldman dye test to examine 14 persons in Chile, finding *Toxoplasma* antibodies in 29 per cent of them. Romaña and Lifschitz (1954) reported the skin test positive in 46 per cent of 72 inhabitants of Tucumán, Argentina, which is situated at 1,460 feet above sea level. In 1956, Varela *et al.*, tested a group of Colombian Indians living at an elevation of more than 8,000 feet and reported 30 per cent positive by the Sabin-Feldman dye test. More recently, Varela and Muñoz Rivas (personal communication) found dye-test antibodies in approximately 13 per cent of a group of sera taken from residents of Bogotá, Colombia.

The present report extends this type of investigation to Central America where, so far as we have been able to ascertain, only one previous study has been made regarding the prevalence of human toxoplasmosis. This was reported by Hoekenga in 1956 and embraced 266 hospital patients from various localities in Honduras, among whom 63 per cent had *Toxoplasma* antibodies as measured by the Sabin-Feldman dye test. (These sera were tested in Feldman's laboratory.)

Through the courtesy of Dr. Nevin S. Scrimshaw, Director of the Instituto de Nutrición de Centro América y Panamá, we were able to obtain three groups of sera from Guatemala and Costa Rica for testing at our Memphis laboratory. The findings from this study are given in the present report. We are indebted to Dr. Scrimshaw and his staff, and to Dr. Frederick J. Stare and Dr. D. Mark Hegsted of Harvard University School of Public Health, for their kindness in making these sera available to us.

METHODS AND MATERIALS

The technique used for testing was that originally described by Sabin and Feldman (1948) for determination of *Toxoplasma* antibody by the methylene-blue dye test. Sera were tested undiluted and at progressive four-fold dilutions (1:4, 1:16, 1:64 etc.) until the end point was reached. Frequent comparative tests on unknown sera by our laboratory and several other research laboratories in the United States have always shown good agreement, indicating that our methods are reliable.

The first group of sera tested in the present study came from 100 Indians of Mayan stock, residents on a cattle ranch near Escuintla, Guatemala. Approximately equal numbers of males and females, ages 16 to 70 years, were included. The ranch, known as Finca San Luis, is situated at an elevation of about 1,000 feet above sea level, near the upper limit of the Pacific coastal plain where the terrain begins to rise abruptly to form the southwestern escarpment of the Sierra Madre. The moderately hot climate is tropical, with heavy rainfall. There are marked wet and dry seasons, but the temperature varies little throughout the year. In addition to cattle ranches, the region supports plantations of sugar cane, bananas, and other tropical fruits. Most

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of the inhabitants of the area are Mayan Indians who form the labor force of the various plantations. They live and work in a rural environment under poor conditions of sanitation. Those tested comprised all available adults among the permanent residents of Finca San Luis, and we believe them to be representative of the population of that region of Guatemala.

A group of 30 Guatemalan military recruits was likewise tested. These were males, ages 15 to 26 years, mostly of Mayan Indian stock. They came from various localities in Guatemala but chiefly from the central highlands where the average elevation is more than 5,000 feet. This region is basically a high plateau, but it is everywhere dissected by deep valleys and modified into innumerable ridges and slopes, so that relatively little tableland remains. The climate differs somewhat from one locality to another, but in general is temperate with annual mean temperatures around 60° F. There is considerable variation in temperature, with minima of around 35° F and maxima of around 85° F, but the extreme cold of more northern temperate climates is not encountered. Rainfall is generally scanty in the area and this fact, together with the cool temperatures, prevents the abundant growth of vegetation found at lower elevations. Many of the inhabitants of the region are pastoral, but there also are towns and villages. The people travel a great deal throughout the area, and many of them migrate to other areas as agricultural workers during the harvest season, so that they are exposed to a variety of environmental conditions. The limited data available concerning the military recruits tested in this study, as well as the small number of sera tested make it difficult to know to what extent our sample is representative of the young men of this area.

The third group tested consisted of 156 persons from the region around Turrialba, Costa Rica.

TABLE 1

Comparative prevalence, by age, of Toxoplasma *antibodies (all titers) in Central America and rural U. S. A (Both sexes combined)*

Source of sera	Age in years						Totals
	15–19	20–29	30–39	40–49	50–59	60+	
Guatemala (Escuintla) (Elev. 1,000 ft.)							
No. tested	11	31	23	15	15	5	100
No. positive	10	26	23	15	15	5	94
Per cent positive	90.9	83.9	100.0	100.0	100.0	100.0	94.0
Guatemala (Mil. Recruits) (Elev. mostly above 5,000 ft.)							
No. tested	13	17	—	—	—	—	30
No. positive	5	10	—	—	—	—	15
Per cent positive	38.4	58.9	—	—	—	—	50.0
Costa Rica (Turrialba) (Elev. 1,800 ft.)							
No. tested	—	40	31	35	29	21	156
No. positive	—	34	25	32	28	19	138
Per cent positive	—	85.0	80.7	91.4	96.6	89.5	88.5
U. S. A. (Tennessee) (Elev. 400 ft.)							
No. tested	135	39	41	38	24	40	317
No. positive	22	20	17	14	12	26	111
Per cent positive	16.3	51.3	41.5	36.9	50.0	65.0	35.0

Approximately equal numbers of men and women were tested, and their ages ranged from 20 to 70 years. These were mostly rural people, chiefly of Caucasian origin, living along the upper limit of the Caribbean coastal plain about 1,800 feet above sea level. This is a hot, humid region with abundant rainfall and heavy growth of tropical rain forest. The sample used in the present study was taken at random and probably is representative of the population of this region.

RESULTS

Table 1 compares the prevalence of positive sera in both Guatemalan samples, in the Costa Rican sample, and in a rural United States sample which we reported recently (Gibson *et al.*, 1956). Since there was little difference in prevalence of antibodies between males and females in any of these groups, data for the two sexes have been combined.

In the Guatemalan lowlands, where we tested 100 persons of both sexes and all ages above 16 years, the overall prevalence was 94.0 per cent. To our knowledge, this is the highest rate yet reported for any area of the world. Antibodies were much less common among the 30 Guatemalan military recruits whom we were able to test. Overall prevalence in this group was only 50.0 per cent. The Costa Rican sample, consisting of 156 sera from both men and women of all ages above 20 years, showed an overall prevalence of 88.5 per cent.

For purposes of comparison, Table 1 includes data for 317 sera collected from a rural United States population of men and women 15 years of age or older. Overall prevalence of positive sera in this group was only 35.0 per cent. Thus it is evident that *Toxoplasma* antibodies, and hence infection with *Toxoplasma*, occur much more frequently in certain parts of Central America than in the area near Memphis, Tennessee, where our United States sample was taken.

Figure 1 compares graphically the proportion of positives in our three Central American samples with respect to the age of the population. The highest prevalence was found in sera from the Guatemalan lowlands, where all persons above age 25 gave positive reactions. The Costa Rican sample likewise showed a high prevalence, but not quite as high as that from the Guatemalan lowlands. The sera from military recruits, restricted to young men 15 to 26 years

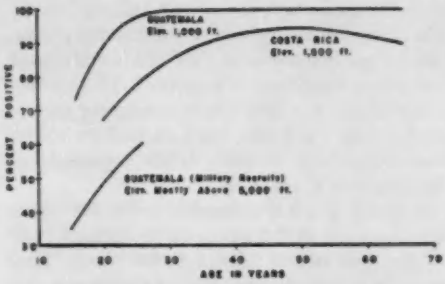

FIG. 1. Comparative prevalence of *Toxoplasma* antibodies in Central America with respect to age in the three groups tested. The curves are freehand approximations based on the prevalence rates for each year of age.

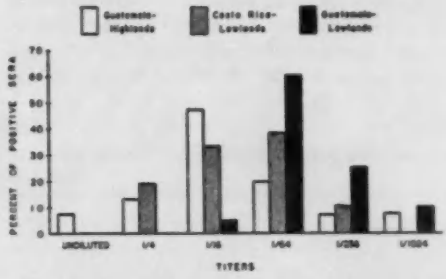

FIG. 2. Comparative frequency of the various dye-test titers in the groups tested. (Males, 15 to 29 years old).

of age, showed a remarkably lower percentage of positives than sera from persons of corresponding ages in the other groups.

The difference between the three population groups which we tested was reflected not only in the prevalence rate for *Toxoplasma* antibodies, but also in the modal titer, as shown in Figure 2. Here, only males of similar ages have been compared, since both prevalence and modal titer are related to age. The data show that in sera from the Guatemalan lowlands the modal titer was 1:64, with very few low titers and a small proportion of negatives, as we have previously indicated. The Costa Rican sample, from a slightly higher elevation, showed a tendency toward a somewhat lower modal titer with a considerable number of sera positive at low dilutions. The military recruits, comprising men mostly from elevations above 5,000 feet, showed a modal titer

of 1:16 with a very high proportion of negative tests.

DISCUSSION

The data given above suggest a striking difference in prevalence of *Toxoplasma* antibodies between inhabitants of the Guatemalan and Costa Rican lowlands, on the one hand, and residents of the Guatemalan highlands, on the other hand. If the sera we studied were truly representative of these areas, this difference would have a high degree of statistical significance (with a probability value of 0.002) and would suggest that perhaps here is a clue to the transmission of the infection. The sera from the two lowland areas would appear to represent well the populations of those regions, since each group was large enough to minimize sampling errors and was drawn at random from a single locality where the population appeared to be relatively homogeneous. Unfortunately, the same degree of reliance cannot be placed on the sample of Guatemalan military recruits, because this group of persons was small in number and was drawn from a relatively large area with much variation in elevation and some variation in cultural patterns. However, it is interesting and perhaps significant to note that, among the 21 recruits for whom exact localities of residence were known, all three who came from elevations below 4,000 feet had positive dye tests, while among the 18 men who came from elevations above 6,000 feet only six had positive tests. This finding strengthens the idea that antibodies were less frequent at higher elevations, and leads one to ask what factors might account for such a difference.

Some evidence has been published to indicate that prevalence may be related to climate. Thus, Gard (1951) found positive skin tests in 45 per cent of 1,894 residents of Stockholm, Sweden, while only 10 per cent of 173 adults from northern Sweden gave positive reactions. Feldman (1953) and Feldman and Miller (1956) reported a series of studies using the dye test, in which the prevalence of *Toxoplasma* antibodies increased fairly regularly from Arctic climates to the tropics. Thus, they detected no antibodies in an Alaskan population; in Iceland, about 11 per cent of the group tested had positive titers (1:16 or above). In the United States, four cities were sampled, with results as follows: Portland, Ore., 17 per cent; St. Louis, Mo., 26 per cent; New Orleans, 31 per cent; and Pittsburgh, 35 per cent. (The latter figure is the only deviation from an orderly progression from cold to warm climates.) Continuing toward the tropics, Feldman and Miller found the prevalence rate of *Toxoplasma* antibodies to be 36 per cent in Haiti, and 68 per cent in Tahiti. With the single exception of Pittsburgh, these results suggest that infection occurs more frequently as one goes from cold to warm climates.

Our data would appear to fit this theory. Both the Costa Rican sample and the sample from Finca San Luis in Guatemala came from areas having tropical climates, and both showed very high prevalence rates as well as a tendency to produce high titers. The Guatemalan military recruits, on the other hand, coming chiefly from an area with more temperate climate, had fewer positive sera and a tendency to produce lower titers. Varela *et al.*, (1953), in discussing some of the results from various Mexican studies, suggest that similar differences found in that country are correlated with climatic variations. While this is an attractive hypothesis, sufficient data are not yet available to establish the point.

These differences are highly provocative, but we do not yet know whether climate, or perhaps some other factors, may be responsible for them. One can only suspect that those factors will reflect the means by which the disease is transmitted to man. With this in mind, it is clear that more detailed studies along these lines are highly desirable to clarify this intriguing feature in the epidemiology of toxoplasmosis.

SUMMARY

Blood sera from 100 persons from a plantation near Escuintla, Guatemala (elevation about 1,000 feet above sea level) were tested for *Toxoplasma* antibodies by the Sabin-Feldman dye test. Males and females, ages 16 to 70, were included. All sera from persons above 25 years of age gave positive reactions. The overall prevalence was 94.0 per cent.

A group of 30 Guatemalan military recruits was likewise tested. These were males, ages 15 to 26 years, from various localities in Guatemala but chiefly from the central highlands (elevation 5,000 feet or higher). The overall prevalence of positive tests was 50.0 per cent.

Similar tests were done on 156 sera collected at Turrialba, Costa Rica (1,800 feet elevation). Donors were males and females, ages 20 to 70 years. The overall prevalence of positive sera was 88.5 per cent.

Comparison of males, 15 to 29 years of age, from the three test groups indicated a tendency for lower titers to be produced at higher altitudes. The possibility is discussed that transmission may be more frequent in warm climates than in cold. The present data seem to fit such an hypothesis, since sera from the Guatemalan and Costa Rican lowlands showed much higher prevalences of positive sera and higher titers than did the sera from the Guatemalan military recruits, who came chiefly from higher elevations. However, sufficient data are not yet available to test this relationship adequately.

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Simulium and Onchocerciasis in the Northern Territories of the Gold Coast, by G. CRISP, B.Sc., Ph.D., F.Z.S., F.R.E.S., Director, Entomological Unit British Empire Society for the Blind, with a foreword by SIR STEWART DUKE-ELDER, K.C.V.O., D.Sc., LL.D., M.D., F.R.C.S., Chairman, Medical Advisory Panel, British Empire Society for the Blind, Director of Research, Institute of Ophthalmology (University of London). 1st ed. 171 pages, illustrated. London, H. K. Lewis and Co., Ltd., 1956. £2.17.6d net.

This handsomely produced small book is essentially a summary of reports of field observations carried out over a period of more than two years, on the distribution and bionomics of *Simulium damnosum* in the Northern Territories of the Gold Coast (Ghana). It contains a suggested control scheme for the fly, based on these observations. While some valuable data are presented on the hydrography of the territories and the specific breeding sites utilized by *S. damnosum* during the seasons of observation, little new information is added to the meager knowledge of the bionomics of the adult. From the data presented in this book it appears that the majority of the numerous and varied field experiments are inconclusive; it is not even clear that *S. damnosum* is the only vector involved. It might be questioned therefore whether the suggested control of the immature stages of *S. damnosum* would be the most effective and economical approach to the control of onchocerciasis in the area studied.

A short introductory section on the pathogenesis of the disease attempts the presentation of a great deal of information in a few pages, with the result that several highly controversial statements appear as fact. Little is said about onchocerciasis as it affects the human population of the area, and nothing new is added in this regard. It is unfortunate that the section on prevention and control of onchocerciasis is wholly devoted to a discussion of measures for control of the fly, and that no mention is made of the possible use of diethylcarbamazine or other drugs, to break the chain of transmission at the human host.

J. N. BELKIN
E. K. MARKELL

The Infectious Diseases of Domestic Animals, 3rd edition, by WILLIAM ARTHUR HAGAN, D.V.M., D.Sc., Professor of Bacteriology and Dean of the Faculty; AND DORSEY WILLIAM BRUNER, D.V.M., Ph.D., Professor of Bacteriology, New York State Veterinary College, Cornell University. Comstock Publishing Company, Inc., Associated with Cornell University Press, Ithaca, New York, 1957, 968 pp.

On pages 535 and 536 of the first volume of this Journal, published in 1952, this reviewer stated, relative to the 2nd edition, that "The Infectious Diseases of Domestic Animals by Hagan and Bruner is highly recommended for students as a text and reference book in microbiology of infectious diseases of animals, and to others interested in various phases of these diseases. It is also a useful and ready reference for those interested and concerned with human infectious diseases, especially those transmitted from animals." The reviewer considers the 3rd edition worthy of similar strong recommendations and high praise. In the latest edition there are no important changes in the arrangement and treatment of the seven parts and 46 chapters of the text. A large volume of new and important information has been reported in the literautre since the earlier edition was published.

In 968 pages of text, illustrations, references, and index, the book contains as much as could possibly be included and make it as lucid and as readable as Hagan and Bruner have done. There are a few more illustrations and more references in this than in the earlier edition. Despite these additions, the material has been so handled that in general it gives to each item of each disease the proper proportion and emphasis in space and importance, and the authors have found space to include a large amount of new additions to the knowledge relative to the infectious diseases of animals. This was mostly accomplished by reconstructing and shortening or omitting portions of earlier text. One wonders, however, how long the ever-increasing knowledge in the field of infectious disease can be contained within one volume.

The reviewer expected to find more space devoted to certain areas; other readers no doubt will have similar feeling about other portions and sections.

The reviewer was somewhat disappointed by the absence of any discussion or reference to the strong leaning toward the acceptance of the newer classification and nomenclature for the important pleuropneumonia group of organisms. This group has been receiving considerably increased attention by American and foreign microbiologists and pathologists. Several of the investigators who have contributed significantly to newer knowledge

on this group of organisms have been using *Mycoplasma* as the generic designation for this group of organisms, and have been arguing for its acceptance. Hagan and Bruner use *Mycoplasma* once. A brief discussion or mention of it, and more references to this new trend, would have added considerably to the very excellent chapter on the pleuropneumonia group in the book. Probably there was not enough published on this at the time the text was submitted to the publishers.

Hagan and Bruner's Infectious Diseases of Domestic Animals is "tops" in its field and no doubt will be well received by those interested in various phases of this important section of medical science.

J. TRAUM

Biology of the Treponematoses, by THOMAS BOURNE TURNER, Professor of Microbiology, Johns Hopkins University, Baltimore, Md., U. S. A., AND DAVID H. HOLLANDER, Assistant Professor of Microbiology, Johns Hopkins University, Baltimore, Md., U. S. A. Based on Studies Carried Out at the International Treponematosis Laboratory Center of the Johns Hopkins University under the Auspices of the World Health Organization. World Health Organization Monograph Series No. 35. World Health Organization, Geneva, 1957.

With the recognition of the fact that the treponemal diseases are a major world health problem, this monograph on the Biology of the Treponematoses is an outstanding contribution to medicine.

The fundamental biology of the treponematoses is surveyed in the first six chapters which discuss the treponemal strains investigated and the methods of isolation; pathogenesis in laboratory animals; in vitro characteristics of the organisms, the immune reactions to treponemes; and the in vitro and in vivo effect of drugs.

Interesting comparisons of the various strains and species of treponemes are made in the next three chapters. Comparisons of disease produced in laboratory animals, immune responses and antigenic relationship, and response of various strains to penicillin were studied in an attempt to establish strain identity.

The authors have thus demonstrated close relationship of treponemal strains from syphilis, yaws, bejel and other endemic syphilis syndromes, particularly as to drug response, immune reactions, and pathogenesis. They feel that certain differences were observed, however, in the rabbit lesion invoked, in the disease response of the hamster, and some immunological patterns as determined by challenge inoculation of rabbits. Serological tests with treponemal and nontreponemal antigens failed to demonstrate any strain differences of consequence.

The authors' failure to establish the treponemal disease pinta in laboratory animals handicapped any comparison of this strain with the others studied.

Commendable features of this monograph are the extensive references to the literature on each subject, generous use of tables on study material and experiments, and color plates and photographs of animal lesions. The excellent descriptions of laboratory animal disease and the study methods used are also commended.

References to "standard serological tests (STS)" however, may be interpreted by the reader that lipoidal antigen tests are "standard" and other tests no "standard." It is recommended that "STS" be considered a broader term designating "Serologic Test for Syphilis" with division into two categories of (1) *treponemal antigen tests* and (2) *nontreponemal* antigen tests.

The final chapter summarizing the investigations reported in the first nine chapters can be read with profit by any worker in this or related fields.

WILLIAM J. BROWN

Acta Leidensia, ed. by the School of Tropical Medicine, Instituut voor Tropische Geneeskunde, Leiden, The Netherlands. Vols. XXVI-XXVII, 218 and 128 pages, bound together. Illustrated. University Press, Leiden, 1957.

The first volume contains 29 miscellaneous articles of which 16 are in English. Of interest are Dinger's discussions of alastrim, and the immunologic overlap in dengue and yellow fever; and van Thiel's and Laarman's papers on *Toxoplasma*. The second volume is entirely devoted to a pilot project of insecticide control of the *A. punctulatus* group (i.e. *farauti*, *koliensis* and *clowi*) and *A. karwari* in Netherlands New Guinea, which had greater success than anticipated, in view of the exophilic habits of these anophelines. This gives a full account of the vectors and of the holoendemic they cause.

L. W. HACKETT

Public Health in Indonesia, by DR. J. LEIMENA, Minister of Social Affairs. The Hague, Djakarta, Bandung, Semarang, Surabaja: N.V. v/h G.C.T. Van Dorp & Co., 1956. 170 p.

In a country where the ratio of doctors to the population is 1:55,000, the contrast between what exists and what is needed in public health is rather overwhelming. In fact, it may be assumed that the public health program in Indonesia must start almost from scratch.

Dr. Leimena has compiled with great precision and care a budget of needed skills, personnel and funds which he has presented against an equally precise outline of those already in existence. A chapter is devoted to each component of the public health program: environmental sanitation; mother and child welfare (no mention is made of a need for family planning); improvement of nutrition both quantitatively and qualitatively; control of yaws, trachoma, tuberculosis, hookworm, plague, typhoid, malaria, leprosy, V.D.; medical care (there are 8 beds per 10,000 people); laboratory services; vital statistics; training of para-medical personnel; health education. The scope of the program is graphically described in a set of ten appendices, each with relevance to a particular chapter in the text.

The author's emphasis on combining health work with other socio-economic activities gives a social framework to the Indonesian public health program which some older systems have not had in the initial stages of planning. Of particular relevance is his point that "In training health personnel, it is deemed necessary to provide some knowledge on *sociology*, which also includes teaching of the existing religious trends in Indonesia, as well as their significance to the public." This is accented by the complex dichotomies in the Indonesian social structure between urban and rural as well as literate and illiterate population groups as briefly described in the study.

Special problems of administration arise in a setting which necessitates extensive localized activity within an overall plan. The author also points out that the red tape of inter-Ministerial administration and consequent overlapping of jurisdiction are both caused and complicated by the necessity to spread the financial burden rather thinly.

Realistic to the point of discouragement, this study nonetheless provides a starting point—in fact many—for the implementation of a truly impressive program.

CONSTANCE A. FREYDIG

Fundamentals of Microbiology, by MARTIN FROBISHER, Sc.D., Formerly Chief, Bact'y. Sect., Communicable Disease Center, United States Public Health Service; Professor and Head, Department of Bacteriology, University of Georgia. 6th edition, 617 pages, illustrated. Philadelphia: W. B. Saunders Company, 1957. $6.50.

A sixth edition over twenty years spells acceptance, whatever the degree of approval a reader may give. Frobisher's book has been successful, and for reasons now more cogent than ever. He has had to choose significant trends among the many, and is acquiring expertness in choice. He has acquired a consciousness of difficulties, and has done a thorough job of revising. He has become a sensitive listener to criticisms, with a dichotomous result. The book has an amazing coverage of subject matter, so that few will find errors of omission of consequence; by the same token, possibly concessions to popular opinion polls have caused the author to scatter his program for basic microbiology rather widely. Basic training, techniques, and purely practical applications are all pooled, and not badly, save for those who prefer stress on one or the other of this triad.

Except for a short chapter on viruses, the text is one of the few that, even nowadays, might well be called "Bacteriology." Mycology and protozoology are briefly included, so the more popular and ordinarily more nearly descriptive term, "Microbiology," used in the title is debatable. However, bacteriology led the way to microbiology and bacteriology is presented broadly, with a fine clarity of English, never too patronizing in tone, and without too many clichés. Perhaps such antiquated and misleading items as the phenol coefficient or occasional lapses into excessive teleology are still with the book only because the author fears to offend someone.

One microbiologist, not given to flattery, called this edition "lively, interesting, and stimulating." It is good, and deserves its popular position, one which it should maintain easily. Under a good teacher, the breadth of scope and wealth of detail should lead to a broad understanding, with care kept on a basic level. Under the meticulous type of teacher, students might well be sunk in facts, but surely in microbiology working from facts to understanding is preferable to generalizations alone.

MAX S. MARSHALL

Biochemical Disorders in Human Disease, edited by R. H. S. THOMPSON, M.A., D.M., Professor of Chemical Pathology, University of London (Guy's Hospital Medical School) AND E. J. KING., Ph.D., D.Sc., F.R.I.C., Professor of Chemical Pathology, University of London (Postgraduate Medical School). 1st edition, 843 pages, illustrated. New York: Academic Press, Inc., 1957. $12.50.

In their preface to this book the Editors acknowledge that the interdependence of the fields of biochemistry and medicine is becoming increasingly apparent. For this reason it seemed to them desirable to prepare a book which would "assemble, relate, and interpret the known facts concerning the biochemical disorders that underlie

or are associated with human disease." They and their collaborating authors have succeeded admirably. The subjects chosen for discussion are those which most readily lend themselves to a biochemical approach. The first chapters deal with diseases of the gastro-intestinal tract and of the liver and biliary tract. These are followed by chapters on anemias, diseases of the blood and the clotting mechanism, and on hypertension. In subsequent chapters, diseases of the kidney and genito-urinary tract, of the nervous system, and of muscle are discussed. Endocrine topics are dealt with in chapters on diseases of the adrenal, diseases of iodine metabolism, diseases of bone and the parathyroid, diseases of the reproductive organs, and diabetes mellitus. A number of miscellaneous disorders of metabolism are also considered. These include abnormalities of amino acid and hemoglobin metabolism, connective tissue disorders, porphyrias, hemochromatosis, glycogen storage diseases and galactosemia, and the lipidoses. There is also a separate chapter on disorders of nutrition.

The authors selected for each chapter include many well-known British and American authorities. Each has done a splendid job in organizing and presenting the facts pertinent to his assignment. The readability of the book is particularly gratifying. Although each topic is treated in a concise manner, the result is much more informative than a simple review. The material presented is remarkably up-to-date and each chapter is accompanied by a very complete bibliography. The book is highly recommended not only for clinicians interested in the impact of biochemistry on medicine but also as a stimulus to biochemists in connection with their own research efforts.

HAROLD A. HARPER

Annual Epidemiological and Vital Statistics, 1954, (Bilingual edition, French and English), 617 pages, World Health Organization, Geneva, 1957. Distributed by Columbia University Press, New York. $10.00.

This seventh yearbook of epidemiological and vital statistics is the most recent in a series of publications which, together with an earlier series of the League of Nations, furnishes international data for the years 1922 to 1954, inclusive. The Introduction points out that the quality of the data varies considerably, but that the efforts of the WHO have resulted in some recent improvements.

The data are presented in three parts: Part I, Vital Statistics and Causes of Death; Part II, Cases of and Deaths from Notifiable Diseases; Part III, Statistics of Health Personnel, Hospital Establishments, and Vaccinations.

Part I contains, in addition to some demographic data on the countries concerned, tables of general vital statistics and tables of deaths by cause, age and sex for 42 countries. There are also several special tables of death rates from selected causes, and a detailed table of infant mortality. This section differs from the corresponding section of the previous yearbook in the greater number of countries covered (42 instead of 35) and in the somewhat greater detail of the tables. One interesting exception is in the table of deaths from tuberculosis by site, sex and age, where only seven categories of tuberculosis are used instead of the previous eleven.

Part II covers 34 notifiable diseases, and includes a table of notifiable diseases by country.

Part III, which is entirely new, contains six tables giving number of physicians and other health personnel, number of hospitals, and number of vaccinations performed against specific diseases, by country.

In comparison with the previous volume, this one has a slightly greater geographical coverage, somewhat more detail, and a wider variety of information.

W. GAFFEY

The Teaching of Hygiene and Public Health in Europe, by F. GRUNDY AND J. M. MACKINTOSH. World Health Organization Monograph Series No. 34, Geneva, 1957, $5.00.

The authors of this monograph review in a very interesting and well organized manner both undergraduate and post-graduate curricula in preventive medicine and public health in 19 European countries. For the factual data the authors have drawn heavily on information furnished by two conferences on medical and public health training organised by the WHO Regional Office for Europe, and have also used material from personal interviews with medical educators in the various countries. The monograph impresses one with its clear and accurate presentation.

The countries dealt with in this report have analyzed carefully and boldly their undergraduate medical curricula. They believe that the medical undergraduate is taught "too much scientific detail and too little of the broad principles and the humanistic basis of medicine in a modern society." Real effort is being made to give the medical student experiences with patients other than in wards and out-patient departments. Homes, factories, mines, and offices are increasingly used as sites for medical education in order to help the student "see and assess a social situation, put himself in

the patient's shoes, and be instrumental in securing for the patient social remedies which are often part and parcel of a comprehensive treatment."

To accomplish this broader humanistic objective, more emphasis in the undergraduate curriculum is constantly being given to medical sociology, statistics and epidemiology. A significant development in several countries is the establishment in undergraduate schools of a Chair of Social Medicine alongside the traditional Chair of Hygiene. The new departments of social medicine are responsible for such areas as vital statistics, demography, epidemiology and psychological and sociological aspects of health and medicine. Departments of hygiene retain their rights over technical hygiene, control of infectious diseases, sanitary engineering and industrial health.

Post-graduate training in public health in Europe is viewed primarily as vocational. Theoretical teaching is at the minimum; emphasis is on the practical problems of health administration with a large amount of time devoted to field trips, demonstrations and periods of in-service training. Public health practice is perceived as being largely team-work and every effort is made to have public health physicians, dentists, nurses, health educators, statisticians, and others, attend class and field work together so that each may become thoroughly familiar with one another's unique contributions.

The monograph concludes with a section which devotes a few pages to each of the 19 countries. Undergraduate and post-graduate training centers are listed; also, for every country a summary curricular statement is provided.

WILLIAM GRIFFITHS

Craig and Faust's Clinical Parasitology, by ERNEST CARROLL FAUST, A.B., M.A., Ph.D., The William Vincent Professor of Tropical Diseases and Hygiene, Department of Tropical Medicine and Public Health, Tulane University School of Medicine, AND PAUL FARR RUSSELL, M.D., M.P.H., Staff Member the Rockefeller Foundation, with the editorial assistance of DAVID RICHARD LINCICOME, B.S., M.D., Ph.D., Howard University, Washington, D. C. 6th edition, 1078 pages illustrated, with 7 in color. Philadelphia, Lea and Febiger, 1957. $15.00.

This 6th edition of an outstanding textbook has been extensively revised, brought up-to-date and new material added. The extensive literature of parasitology has been thoroughly covered making the bibliography one of the most valuable features of the book. As in prior editions the text includes: 1. general introduction and orientation, 2. protozoan infections, 3. helminthic infections, 4. arthropods and human disease, 5. technical appendix. The illustrations are well chosen, numerous and in keeping with the high quality of the book. Each parasite is considered under the headings of history, geographical distribution, morphology, habitat, life cycle, epidemiology, reservoir hosts, pathology, symptomatology, diagnosis, treatment, prognosis and prevention. The control of arthropods is well presented and the use of recently discovered chemical agents is given in detail. The complete coverage of the subject makes this book of great value to zoology majors in college, graduate students, medical students, physicians and public health workers. I recommend it most highly to this group as a very complete and, at present, the most up-to-date book on clinical parasitology.

HAROLD W. BROWN

Zinsser Bacteriology, 11th Edition, by DAVID T. SMITH, NORMAN F. CONANT, JOSEPH W. BEARD, HILDA POPE WILLETT, JOHN R. OVERMAN, IVAN W. BROWN, D. GORDON SHARP AND MARY A. POSTON; Duke University School of Medicine. 953 pages, illustrated. New York, Appleton-Century-Crofts, Inc., 1957.

This classic, the first edition of which was written by Hiss and Zinsser and issued in 1910, has now had another major revision and face lifting. A number of new collaborators have helped Drs. Smith and Conant to bring the text up to date by adding a good deal of new material and by placing the older sections in a new perspective in relation to recent additions to our knowledge in the field. As indicated in the preface to this edition, the most rapid developments have been in the areas of bacterial physiology, immunology and viruses; the sections covering these areas have been completely rewritten and enlarged to accommodate the new material. There is also a new chapter on blood groups and immunohematology. The available information on susceptibility of different organisms to the newer antibiotics was brought up to date and, when information was available, references to the effects of adrenocortical hormones on specific infections were included.

The authors have succeeded in retaining the basic flavor of the original and early editions of Hiss and Zinsser's text which made it so popular among students of clinical medicine and public health, by including, along with descriptions of the biological characteristics of microorganisms, the interesting features relating to the reactions of the tissues to the organisms and their products.

The text is clearly printed on good paper, is well illustrated, contains a number of good summary tables and there are large numbers of refer-

ences following each chapter. This new edition should help retain for this book its position of eminence among the leading texts available for students of medicine and public health.

MAXWELL FINLAND, M.D.

Pharmacology for Medical Students in Tropical Areas, 1st edition, by ROGER A. LEWIS, A.B., M.D., (Johns Hopkins), Formerly Visiting Professor of Pharmacology, Seth Gordhandas Sunderdas Medical College, Bombay, India, The Popular Book Depot, 1957. 524 pages. Rs 15.00.

This book is essentially a teaching manual and should serve as a suitable introduction to pharmacology undergraduates or medical students in tropical areas. It is a well-organized, concise text, reflecting a "running commentary . . . of the author's personal opinion."

The contents were divided in traditional style into: A—General pharmacology, B—Chemotherapy of infectious disease, and C through I—Drugs acting on various systems. Section J, on the prevention or treatment of nutritional deficiencies, is of importance in the tropics. Sections K, L and M contain appendices in practical pharmacy, experimental pharmacology and table of drugs for adults and children. There is an index, but unfortunately no references are cited as documentation of views expressed by the author. To the isolated student with an inquiring mind, this is a real handicap. Fortunately, chemical structures are given and, in the Appendix, the terminology of *Pharmacopeia Internationalis*, published by the W.H.O., is used.

In contrast to the texts used in the U.S.A., some sections are relatively sparse, i.e., the chapter on General Anesthesia (10 pages), which would not serve adequately in the continental U.S.A. The author deserves credit for attempting to compress into this text's pages material which, in current texts in the U.S.A., occupy four times this volume.

HAMILTON H. ANDERSON

World Directory of Medical Schools, 2nd edition, 314 pages. Geneva, World Health Organization, 1957. $5.00.

This is a second edition of the WHO *World Directory*, now brought up to date and enlarged in coverage and scope. More than eighty countries are represented including the USSR, which was almost a complete blank in the former work published in 1953. In addition to the salient facts about each school, such as enrollment, teaching staff, fees etc., the general educational system of the country is outlined with its historical background, and the undergraduate medical training is briefly described. The ratio of physicians to population throughout the world is given in the form of a map, varying from 1:434 in Israel to 1:174,640 in Nepal. The countries are listed in alphabetic order and each is dealt with in a separate chapter; annexes 1 to 7 summarize these data by continent, and annex 8 gives the world totals. Such an analysis, in spite of unavoidable lacunae, is an important step toward a comprehensive view and understanding of the present situation with respect to medical education in the world, and provides an indication of what might be done to further its development.

L. W. HACKETT

BOOKS RECEIVED

The Medical and Veterinary Importance of Cockroaches, by Louis M. Roth and Edwin R. Willis, 147 pages, illustrated. Washington, D. C., Smithsonian Institution, 1957.

Schutzimpfungen, by Heinz Spiess, Gottingen, & others, 356 pages, illustrated. Stuttgart, Georg Thieme, Verlag. Intercont. Book Corporation, New York City, 1958.

Biologie Du Noir, by Georges A. Heuse, 347 pages. Brussels, Éditions Problèmes d'Afrique Centrale, 1957. DM 56, $13.35.

Annual Review of Entomology, by E. A. Steinhaus, 520 pages. Palo Alto, Annual Reviews, Inc., 1958, $7.00.

Non-Venereal Syphilis; A Sociological Medical Study of Bejel, by Ellis Herndon Hudson. 203 pages, illustrated. Baltimore, Williams and Wilkins Co., 1958. $7.00 (U.S.).

NOTICE TO CONTRIBUTORS

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